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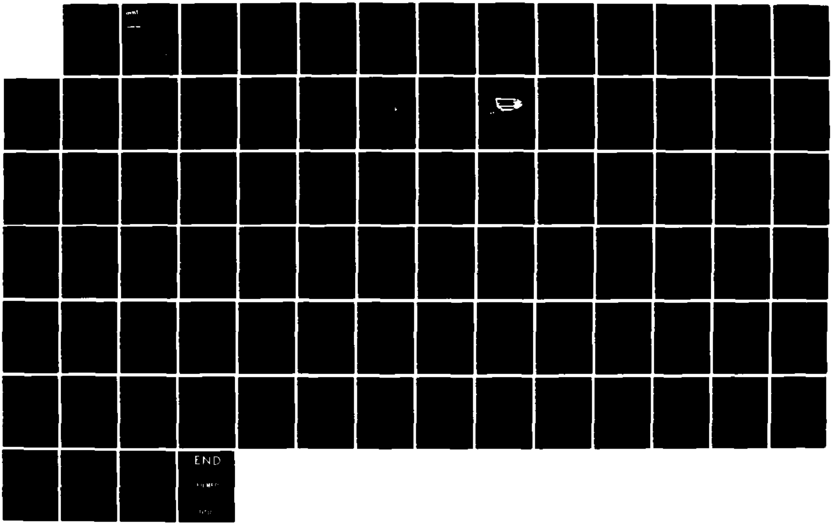

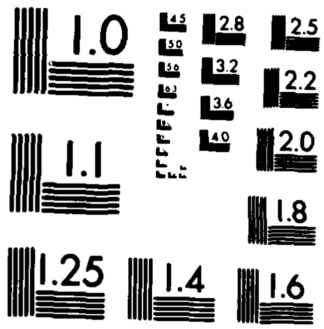

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Chemical Characterization and Toxicologic Evaluation of Airborne Mixtures

Inhalation Toxicology of Diesel Fuel Obscurant Aerosol in Sprague-Dawley Rats

FINAL REPORT,
PHASE 3, SUBCHRONIC EXPOSURES

Simon Lock, Ph.D.
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MAY, 1984

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Biology Division
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Inhalation exposures were performed twice per week, for 13 weeks, to determine whether there was any potential toxicity to rats of comparatively low concentrations of a condensation aerosol from diesel fuel. Animals were divided into 4 groups (24 per sex in each group) and exposed to aerosol concentrations of 0, 0.25, 0.75 and 1.50 mg diesel fuel aerosol/L for 4 hours per day. A fifth group (12 per sex) was used as vivarium controls. Body weight and food consumption were measured weekly for duration of the exposure,

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and also during a two month recovery period. Changes in breathing frequency and the response of animals to a loud sharp sound (startle response) were measured in selected animals prior to the start of the exposures, at various time points during the thirteen week exposure period, and at monthly intervals during the recovery period. Assays were performed on selected animals at the end of the exposure period, and again after the two month recovery period. Endpoints included pulmonary function tests, numbers of alveolar free cells, clinical chemistry, hematology, organ weights and histopathology.

No mortalities were recorded during the exposure or recovery periods. Slight toxicity occurred at these low aerosol concentrations with the loss in body weight of all treated animals during the exposure period. During the exposure period there were also some slight changes in startle reflex, however, these were apparently acute effects, and there appeared to be no permanent CNS involvement as measured by this endpoint. Immediately post-exposure, the numbers of lavaged alveolar macrophages were slightly elevated in all aerosol exposed animals. Pulmonary function tests, pulmonary gas exchange and dynamic lung tests were all apparently unaffected by these low diesel fuel aerosol exposures. Changes in tissue weights in aerosol exposed animals were minor and the few histopathological lesions were randomly scattered amongst all groups included in this study and were more attributable to the age of the animals than any specific treatment group. No significant cumulative toxicity may be attributed to these diesel fuel aerosol exposures.

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Chemical Characterization and Toxicologic Evaluation of Airborne Mixtures

INHALATION TOXICOLOGY OF DIESEL FUEL OBSCURANT AEROSOL IN SPRAGUE-DAWLEY RATS

FINAL REPORT, PHASE 3, SUBCHRONIC EXPOSURES

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EXECUTIVE SUMMARY

Rats of both sexes have been exposed, twice per week, for 13 weeks, to low concentrations of a condensation aerosol from diesel fuel. These inhalation exposures were performed to determine whether there was any potential toxicity from comparatively low aerosol concentrations. Animals were divided into 4 groups (24 per sex in each group) and exposed to aerosol concentrations of 0, 0.25, 0.75 and 1.50 mg diesel fuel aerosol/L for 4 hours per day. A fifth group (12 per sex) was used as vivarium controls.

Body weight and food consumption were measured weekly throughout the thirteen weeks of exposure and also during a two month recovery period. Changes in breathing frequency and the response of animals to a loud sharp sound (startle response) were measured in selected animals prior to the start of the exposures, at various time points during the thirteen week exposure period, and at monthly intervals during the recovery period. At the end of the exposure period, and again after the two month recovery period, sub-populations from each group were used in a series of assays. These assays included pulmonary function tests, numbers of alveolar free cells, clinical chemistry, hematology, organ weights and histopathology.

No mortalities were recorded during the exposure or recovery periods; however, the loss in body weight of all treated animals during the exposure period shows that there was some slight toxicity occurring at these low aerosol concentrations. This toxicity was evidently reversible since the weights of treated animals increased during the recovery period at a rate that was more rapid than that of the sham exposed control animals.

During the exposure period there were some slight changes in startle reflex, however, these were apparently acute effects, and there appeared to be no permanent CNS involvement as measured by this endpoint. Changes in the respiratory system were also considered minor. Also during the exposure regimen there were no changes in respiratory frequency that were exposure related. Immediately post-exposure, the numbers of lavaged alveolar macrophages were slightly elevated in aerosol exposed animals, but there were no clear concentration related effect. In the pulmonary function tests there were some trends towards smaller lung volumes in animals exposed to diesel fuel aerosol; however, there were no significant concentration-related effects. Pulmonary gas exchange and dynamic lung tests were also apparently unaffected by these low diesel fuel aerosol exposures.

Changes in tissue weights as a result of diesel fuel aerosol were minor, and the only histopathological lesions recorded were minor and randomly scattered amongst all groups included in this study. Thus these lesions are most likely age-related rather than resulting from aerosol exposure.

It has been concluded that there is no significant cumulative toxicity which may be attributed to the diesel fuel aerosol exposures described in this report.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Uses of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

The authors would like to thank the following persons for competent assistance during this study: Dr. Cindy Morse for assistance with the necropsies, William Klima and Fred Stenglein who provided the essential technical support, Richard Davis for animal husbandary, and Violet Wright for preparation of histology slides.

Aerosol support and analyses of collected chamber samples were carried out under the direction of Drs. Mike Guerin, Bob Holmberg, and Roger Jenkins by Dr. Doug Goeringer, Tom Gayle, and Jack Moneyhun.

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INTRODUCTION

Battlefield smokes and obscurants are used by the armed forces to assist in defending men, material, and installations against observation and bombardment. Because of their ability to degrade the performance of target acquisition and guidance devices, conceal friendly ground maneuvers, deceive the enemy, and provide a means of signalling and marking, smokes and obscurants will be widely employed in the event of hostilities, and are increasingly being used in training in order to create realistic battlefield conditions. The U.S. Army Medical Bioengineering Research and Development Laboratory is actively investigating the toxic properties of various smoke/obscurant munitions and systems to estimate their potentials for adversely affecting the performance capabilities of soldiers in combat, for causing immediate or delayed health effects in troops exposed in training and for affecting the health, safety, and comfort of persons engaged in the manufacture of smoke munitions.

One material widely used as a visual obscurant is an aerosol generated from diesel fuel. When injected into the exhaust manifold of a tactical vehicle, diesel fuel instantly vaporizes, is expelled with the vehicle exhaust, and upon exiting the exhaust system condenses to form a dense white "smoke" which rapidly provides a large and effective screen for the vehicle and supporting troops. Since there is a potentially large population at risk, and because little information is available on the potential health and performance effects of exposure to diesel fuel in this form, a number of studies have been designed to expand the available data base so that appropriate health protection decisions may be made. Inhalation exposures of rodents have been conducted to determine the biologic effects of exposure to variations in aerosol concentration, duration of each exposure, frequency of exposures and total number of exposures.

The first phase was a series of acute, range-finding experiments to establish the maximum tolerated concentration for a given exposure duration (1). Since both concentration (C) and duration of exposure (t) were variables, data on the relationship of mortality to the Ct product were also acquired. This product of concentration of airborne contaminant and time of exposure has often been used as an index of the "dose" of material delivered to the body and therefore the exposure conditions required for a specific effect (2). This relationship is not always valid and must be used with caution.

Mortality was found to be highly correlated with the Ct product during single exposures ($p = 0.0001$), and 83 percent of the variation in mortality was explained by the Ct product (1). Under these circumstances it was considered justifiable to use the Ct product to estimate maximum tolerated exposure conditions to be used in repeated exposures. A probit analysis (3) was used to relate mortality to the log of the Ct product in order to estimate the Ct product which on the average induces 1% mortality. This estimated value was 12.2 mg·h/L and the 97.5% lower confidence bound of this value was calculated to be 8.2 mg·h/L.

A matrix design of repeated exposures was used in Phase 2 (4). A Ct product of 8 mg·hr/L was employed as a lower exposure regimen and 12 mg·hr/L as an upper level at which some mortality might be anticipated. Exposure variables were the Ct product, frequency of exposures (1 or 3/wk), duration of exposure (2 or 6 hr), sex, and time after last exposure (2 days or 2 weeks). All groups received a total of 9 exposures regardless of frequency of exposures. Thus groups exposed once per week were treated for 9 weeks; those exposed 3 times per week were assayed after 3 weeks of exposure. Assays performed on the animals were chosen on the basis of anticipated effects. They included pulmonary free cell number and phagocytic activity, pulmonary function, neurotoxicity, clinical chemistry, blood cell number, organ weight, and histopathology. All assays were performed within 2 days after the last exposure and after 2 subsequent weeks without exposure.

After exposure, the primary target organ was the lungs. Focal accumulations of pulmonary free cells were observed in the lung parenchyma, associated with thickening and hypercellularity of alveolar walls. The number of lavaged pulmonary free cells correlated well with histologic observations, remaining elevated after two weeks without exposure. Lung volumes were altered by exposure with an increased functional residual capacity (FRC), decreased total lung capacity (TLC), and decreased vital capacity (VC). There was also a decrease in carbon monoxide diffusing capacity in a number of the exposed groups. Frequency of exposure appeared to be the dominant variable over the range of parameters studied, with exposures 3 times per week being more deleterious than 1/week. Variation in duration of exposure appeared to have little effect and a 'dose'/response was often not apparent with differences in concentration.

Information acquired during the first two phases of this inhalation study was used to design the exposure regime for the third and final phase, a sub-chronic (13 week) study. The purpose of the third phase was to determine the effects of multiple exposures to low concentrations of diesel fuel aerosol. The duration of exposure was set at 4 hours, and all animals were exposed twice per week for a total of 26 exposures. The 4-hour duration of each exposure was chosen as an average time between 2 and 6 hours used in the Phase 2 studies while the number of exposures/week was chosen in an attempt to relate to potential exposure patterns in humans. Exposure variables were concentration of aerosol, sex, and time after the last exposure (within 5 days or after a recovery period of 2 months) that the animals were tested and sacrificed. This report summarizes observations made during exposure and results obtained from a battery of assays at the time of sacrifice.

MATERIALS AND METHODS

Experimental Design

Equal numbers of 8 week old male and female Sprague-Dawley rats were acquired from a commercial source (Charles River Breeding Laboratories, Inc., Wilmington, MA). They were maintained in quarantine for two weeks to ensure that only essentially healthy animals were used in this study. At the end of the quarantine period animals were randomly divided into 4 groups of 24 male and 24 female rats to serve as the three exposure groups and the matched sham-exposed control group. A fifth group comprising 12 male and 12 female rats was used as vivarium controls. Exposures commenced when the rats were 18 to 21 weeks of age. A diagram of the exposure schedule (Figure 1A) and tables showing ages of the animals at various points during the study (Table 1A) and the multiple use of animals (Table 2A) are given in Appendix A.

All animals including the vivarium controls were housed individually in hanging, stainless steel, wire mesh cages. Purina rat chow was provided ad libitum except during exposures. Water was provided using an automatic watering system. In order to prevent the introduction of Pseudomonas aeruginosa (an organism capable of producing pneumonia in animals) by the water supply, it was hyperchlorinated to 16 ppm as it entered the building. The actual chlorine concentration in the water the animals received was in the range of 3-5 ppm; a concentration range that is commonly used in animal facilities to prevent the growth of the bacteria. A 12 hr-on/12 hr-off light cycle was maintained.

Exposure Methods

The exposure chambers and aerosol generation system have been previously described (5). Briefly the generator was designed to model the vehicle exhaust system used by the military to produce smoke from diesel fuel. It consisted essentially of a 1-in.-O.D. stainless steel tube about 1 m long with a Vycor heater fitted into one end. This heater was maintained at 600°C. The distal end of the generator was heated to 350°C by a heating tape. Nitrogen entered the end near the Vycor heater and exited at the opposite end of the tube. Diesel fuel was metered onto the tip of the Vycor heater where it was flash vaporized and carried by the hot nitrogen out of the generator and into the cool supply air entering the exposure chamber. Aerosol concentration in the chamber was controlled by the rate of flow of fuel into the generator at a constant flow rate of air through the chamber.

Exposures were whole-body and performed in 1.5 m^3 New York University style inhalation chambers with rats housed individually in 6 tiers within the chamber. Animals were housed in the inhalation chamber only for the duration of the exposure. Within each chamber animals were rotated on a preassigned schedule so that during the course of the study each animal was moved both in a vertical plane and laterally from left to center to right side of the chamber. Chamber humidity and temperature were maintained at 70% and 22°C ± 2° respectively. Aerosol concentration was monitored continuously by infrared backscatter probes at the top and bottom of the chamber. Particle size was determined by cascade impaction at random intervals during the study. The mass median diameter was 0.43 - 0.75 μm with a geometric standard deviation of 1.4 - 1.5. Actual size varied slightly with aerosol concentration. The percent of fuel in the vapor phase also varied slightly with particle concentration but was on the order of 10.3-13.6% for the concentrations employed in this study. Aerosol distribution within the chamber was uniform (5) and there was no evidence of appreciable particle growth between the top and bottom of the chambers. Periodic filter samples were also taken for gravimetric determination of concentration during each exposure. Some of these filter samples were also analyzed by high performance liquid chromatography and gas chromatography as part of routine monitoring of stability of the fuel (5). All fuel was from one shipment of a standard blended fuel (Phillips Petroleum Co.). The fuel was stored in the Oak Ridge National Laboratory chemical repository at 5°C. When a fresh supply was required for the inhalation study or on a monthly basis, 5 gallons was removed from the storage area, brought to ambient temperature, and then mixed before use. During the time a can of fuel was in use it was held at room temperature in sealed fireproof oil cans.

Observations During Exposure

Body Weight and Food Consumption

Individual records were kept for all animals. All animals were weighed once per week, on Monday. The vivarium control group was used initially to compare rate of growth with that observed in the sham-exposed group. Thus, weighing of this control group commenced at the same time as the weighing of the sham exposed group. Food consumption was determined for 12 animals (6 of each sex) in each treatment group. Food consumption was also determined in the vivarium control group; however, since this had not been in the original protocol, the animals used were only started in the feeding study part way through the experimental period. A large supply of food for each animal was kept in a 1-gallon plastic jar which was refilled as required. Food was taken from these jars to supply a hopper on the side of the animal's cage. Once per week, food in the hopper was returned to the jar and weighed. This method assumed that loss of food from the hopper other than by eating was some constant fraction of food consumed and uniform across all treatment groups.

Additional experiments were performed using 12 male and 12 female from each treatment group (not vivarium controls) to determine whether successive exposure to diesel fuel aerosol had any cumulative effect on breathing frequency or startle reflex. The designs of these two assays are described below.

Breathing Frequency

Chronic exposure to an aerosol could have an effect on the breathing frequency of an exposed animal because significant lung damage could require the animal to breath more rapidly in order to maintain adequate oxygenation of the blood. Each animal selected for this assay was tested prior to the first exposure to obtain a baseline frequency, then before the 14th and 26th exposure, and after one and two months recovery. Animals were confined in approximately 3.5L rigid plastic jars that were tightly capped but had air drawn through them at the rate of 1L/min. After 30 minutes of acclimatization each jar was sealed briefly and the breathing frequency of the animal was recorded using a barometric technique (6). Air in the jar was warmed and humidified during inhalation by the rat so that thoracic expansion was greater than the volume of air inhaled from the jar. The net effect was an increase in pressure within the jar, which was measured using a differential transducer (Validyne DP-45).

Startle Reflex Assay

The startle reflex assay was used to test the time to reaction and the force of response when rats were startled by a sharp auditory stimulus. Rats were placed in a wire box within a larger sound-insulated box (Fig. 1). A constant white noise at 85 dB within the larger box helped eliminate outside noises. After an acclimation period of 10 min., rats received a series of five 10 msec pulses of noise at 13,000 Hz and 110 dB separated by 25 sec. Their response, or startle reflex, was monitored by a Gould load cell under the wire box. The entire procedure, including data acquisition and analysis, was controlled by an Apple microcomputer.

All animals to be held for the two month recovery period were assigned to be tested for startle response at various time points during the study. Both male and female animals were tested prior to the first exposure to obtain a baseline response for the individual animal, in order to permit each animal to act as its own control. Immediately after the first, fourteenth, and twenty-sixth exposure, the males only were tested to determine whether, at the low aerosol concentrations used, there were any acute effects and also, if acute effects were evident, whether there was any evidence of these being additive with a subtle chronic effect. Prior to the fourteenth and twenty-sixth exposure, and after one and two months recovery both males and female animals were tested to determine whether there were any chronic effects that were resolved during the postexposure period.

Previous experience with this assay (4) has shown that the data can best be analyzed as differences between pre-exposure and postexposure values for each individual. Thus each animal served as its own control.

Observations Following Exposure

Pulmonary Free Cells

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg), the abdomen was opened, and the animal was killed by aortic bleeding. The diaphragm was then cut to collapse the lungs, and the upper trachea was exposed and cannulated with polyethylene tubing (PE205). Lungs were then lavaged with phosphate buffered saline (PBS) at room temperature; volumes used were approximately 36 percent of the estimated vital capacity, based on body weight. The first lavage remained in the

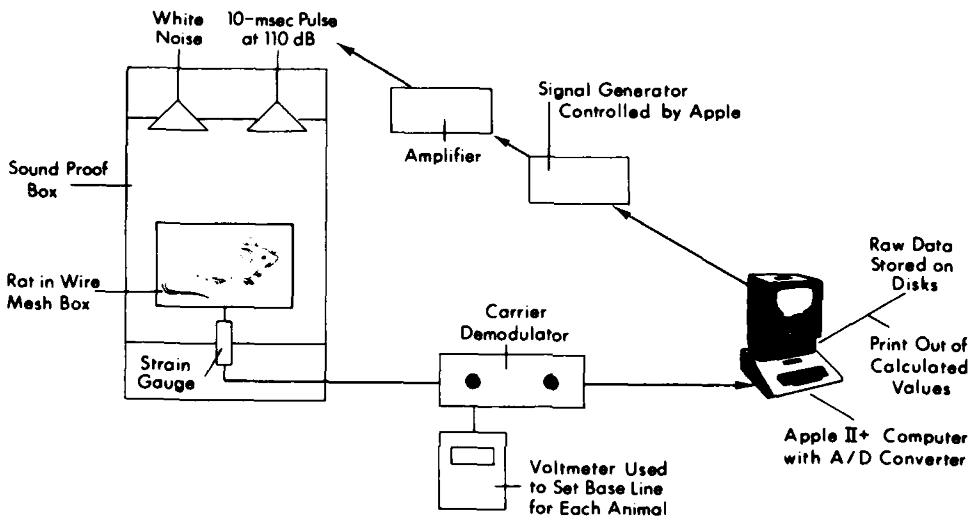

Figure 1

Schematic diagram of apparatus used for startle reflex assay.

lung for 2 minutes before withdrawal; five subsequent washes were performed without waiting between injection and withdrawal.

Lavaged cells were kept on ice, centrifuged twice in refrigerated PBS and resuspended in 6 mL of PBS. Total alveolar cell and macrophage counts were performed on a hemocytometer. Cell viability was determined by trypan blue exclusion.

Clinical Chemistry

Before rats were used for lung lavages, blood was taken from them for clinical chemistry. Blood was also taken from some of the animals used for pulmonary function tests at time of sacrifice. Aortic puncture with a 21 g needle was used to draw the blood into both heparinized neonatal sized and non-heparinized standard sized vacutainers. The heparinized blood was used immediately for counts of red and white blood cells on a Fisher Autocytometer. Non-heparinized blood was allowed to clot and was then centrifuged. The serum was removed, frozen in liquid nitrogen and then stored at -5°C until it was assayed for clinical chemistry parameters 3-5 days later. The following were measured routinely:

alkaline phosphatase	SGOT	cholesterol
triglycerides	uric acid	urea nitrogen
glycose	bilirubin	creatinine
sodium	potassium	

Pulmonary Function Tests

Terminal pulmonary function tests were performed on 16 randomly preselected animals (8 per sex) from each treatment group. All tests were performed in a plexiglass body box which could be sealed and used as a whole body plethysmograph (Fig. 2). Three pressure transducers were used for various tests; they were connected to amplifiers on a multichannel Electronics for Medicine electronic recorder. Tracings from these transducers, a nitrogen analyzer, and an electronic integrator were monitored on the oscilloscope of the recorder and recorded on light sensitive paper. All calibrations were performed by standard manipulations of the body box to mimic conditions during each test.

Rats were anesthetized with i.p. injection of 50 mg/kg of pentobarbital. The trachea was exposed and cannulated with a 4 cm length of polyethylene tubing (1.67 mm ID and 2.42 mm OD). The animal was placed on its back in the box. The tracheal cannula was directly connected to the outside of the box through a plastic tubing adapter. An open-ended, water-filled (1.3 mm ID and 2.0 mm OD) cannula was introduced into the esophagus and flushed with water, and its position was adjusted to obtain maximal pressure deflections.

After the box lid was sealed, respiratory flow was measured by a pneumotachograph and a Validyne MP45 differential pressure transducer,

Figure 2

Body box used for pulmonary function tests.

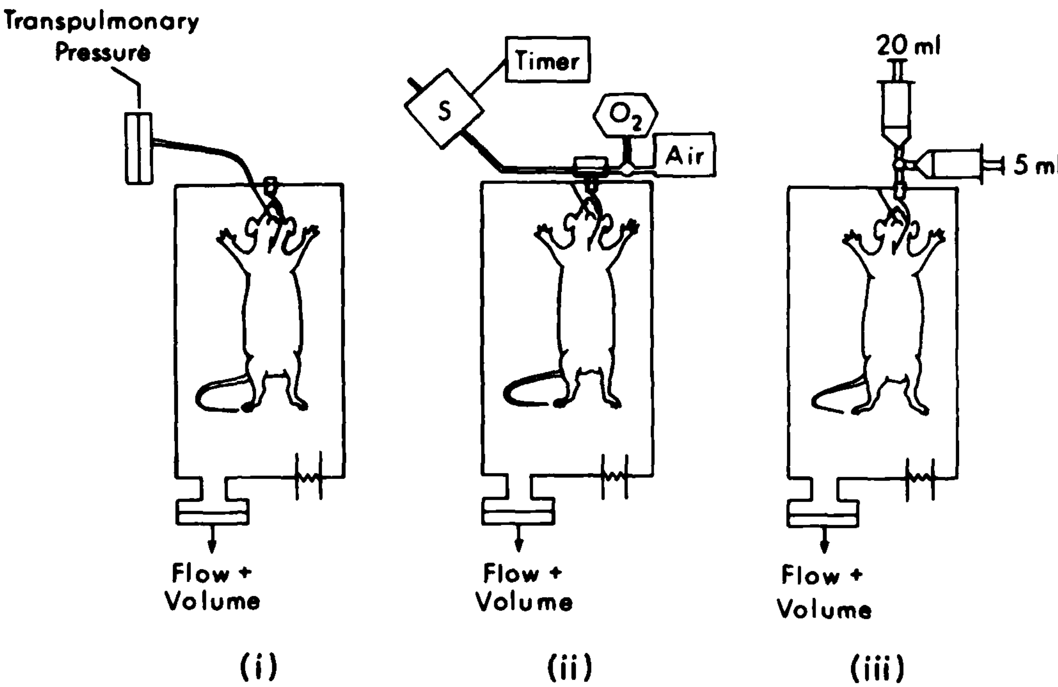

Figure 3

i) Body box configuration during measurement of resistance. Note esophageal cannula connected to differential pressure transducer on box to measure air flow through pneumotachograph.

ii) Body box configuration during multibreath nitrogen washout maneuver. Either air or oxygen flow past opening to tracheal cannula and N_2 analyzer. Lungs are inflated while solenoid (S) is closed.

iii) Body box configuration during measurement of single-breath CO diffusing capacity.

illustrated in Figure 3(i). Flow signals were electronically integrated to provide a volume tracing. Changes in esophageal pressure were recorded from a water-filled Validyne MP45 differential pressure transducer also illustrated in Figure 3(i). Esophageal pressure, respiratory flow, and tidal volume were recorded during spontaneous breathing. Resistance was calculated from these recordings by the method of Amdur and Mead (7), with subtraction of resistance in the tracheal cannula and associated tubing.

The second lung function test was a multibreath nitrogen washout maneuver (8). Air (300 mL/min) flowed past a "T" connection to the tracheal cannula within a small plexiglas block [Figure 3(ii)]. A solenoid on the exit side of the block automatically cycled open and closed, alternately inflating the lungs for 0.5 sec and allowing 0.75 sec for deflation. A constant flow of air resulted in standardized positive-pressure ventilation of the lung. During the nitrogen washout maneuver, the air supply was changed to oxygen by turning a 3-way valve during exhalation. Thus the next inhalation was of 100 percent oxygen. (There was no dead space in the oxygen delivery system.) The probe for the nitrogen analyzer (Hewlett-Packard, Vertek Series) was on the tube between the "T" and the tracheal cannula and thus in position to detect end-tidal nitrogen concentration during several breaths until a concentration of 2 percent was reached.

Single-breath carbon monoxide diffusing capacity was the third pulmonary function test performed. Diffusing capacity simply refers to the volume of gas which can be exchanged across the lungs in a given time. An air mixture containing neon, acetylene, and carbon monoxide was injected into the lungs and then rapidly withdrawn, the last portion of it being kept for analysis of neon and carbon monoxide concentrations. Neon is used as an insoluble tracer to help establish the volume of air in the lungs with which the injected gas mixed. Carbon monoxide was used to determine the rate of diffusion across the lung membranes, with the assumption that the blood was a sink for carbon monoxide (in the concentration range used).

A diagram of the procedure is shown in Figure 3(iii). The body box was used with an assembly of one three-way valve and two plastic syringes. The 20-mL syringe was filled with lung diffusion gas mixture obtained from Matheson Gas Co. (air with 0.4 percent carbon monoxide, 0.5 percent neon, 0.5 percent acetylene). The volume of gas injected into the lungs was the inspiratory capacity which was obtained prior to carrying out this maneuver by injecting air from a 20-mL syringe into the lungs beginning at the end of exhalation. Maximal inflation or inspiratory capacity was the volume injected to give a transpulmonary pressure of 30 cm water. While lung volume changes were being recorded, the gas mixture was rapidly injected into the lungs and immediately withdrawn until only 5 mL remain in the lungs. The three-way valve was quickly switched to connect the 5 mL syringe to the animal and the remaining 5 mL was withdrawn. This 5 mL was immediately taken to a Carle Analytical Gas Chromatograph (Model 111) for analysis of neon and carbon monoxide concentrations. Diffusing capacity was calculated by a standard equation (9).

One of the primary means of detecting damage to the small airways is by maximal flow-volume curves or the flow during maximal forced exhalation

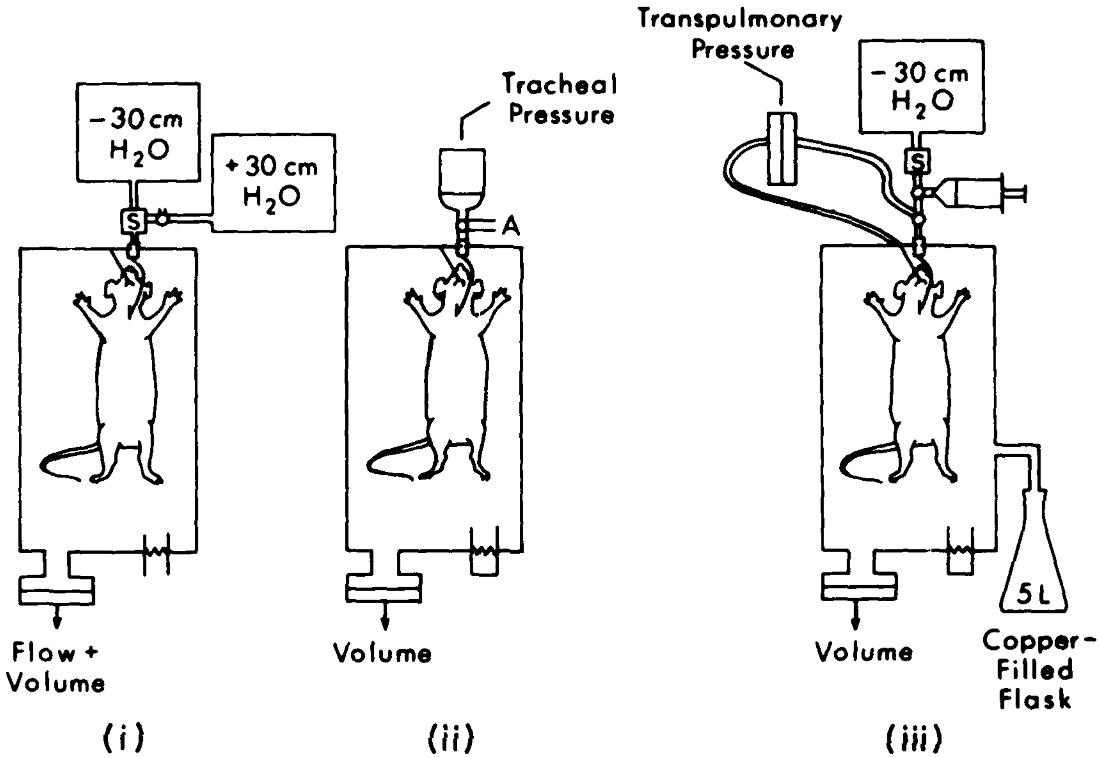

Figure 4

i) Body box configuration during maximal forced exhalation maneuver. Lungs are inflated by pressure reservoir at 30 cm H_2O and then connected by solenoid (S) to subatmospherics reservoir for deflation.

ii) Plethysmograph configuration during measurement of FRC. Opening (A) is occluded at end-expiration. Changes in tracheal pressure and lung volume (plethysmograph pressure) are recorded as animal breathes against closure.

iii) Plethysmograph configuration during quasistatic pressure-volume curve. Lung volume changes are recorded from pressure changes in plethysmograph as lungs are inflated and deflated by syringe. Transpulmonary pressure is difference between esophageal and tracheal pressures. Lungs are taken to -30 cm H_2O by pressure reservoir on opposite side of solenoid (S).

(10). In this procedure, the animal was forced to inhale air to a maximal inspiratory pressure of 30 cm water and was then connected to a reservoir held at -30 cm water to achieve a maximal deflation rate. The pressure reservoirs were 5-gallon glass jugs so that connection to the animal did not decrease their pressure. The connection to the negative pressure reservoir was a three-way solenoid shown in Figure 4(i). The system was designed and tested to assure that it was not lim g flow during forced exhalations.

A three-way valve, shown in the figure, was switched to connect the animal to the positive pressure reservoir. As soon as lungs were fully inflated, the solenoid was switched to connect the animal with the negative pressure reservoir, and flow and volume were recorded during deflation. The body box was used with a pneumotachograph. Volume changes were obtained by integration of the flow signal. At least two forced exhalations were performed and analyzed.

Functional residual capacity (FRC) was the next lung function test and was measured by the Boyles law technique (11). The trachea was occluded at the end of exhalation (FRC), and changes in tracheal pressure and lung volume were recorded as the rat tried to breathe against the sealed tracheal cannula. The cannula was closed at FRC so that air pressure within the lungs was equivalent to atmospheric pressure. Thus known values included original pressure in the lungs, change in pressure during attempted inhalations, and change in lung volume as the animal's lungs expanded. Using Boyle's law the original volume or FRC can be calculated. Atmospheric pressure was recorded daily from a mercury barometer.

The schematic of the system used for FRC is shown in Figure 4(ii). A Statham P23ID pressure transducer was connected by a three-way fitting directly to the tracheal cannula so that the animal was breathing through the one open end, labeled A in Figure 4(ii). The body box was changed into a plethysmograph at this point by closing the valve on the pneumotachograph to increase the sensitivity of measurement of volume changes. At the end of an expiration, opening A was occluded with a finger. Three or more breaths were recorded and the entire procedure was repeated at least 3 times.

The basic intent of the quasistatic pressure volume maneuver, the last test in this battery, was to establish the pressure-volume relationships in the lung _in situ_. This was done in a semistatic or quasistatic manner. The lungs were inflated to a maximal lung volume and then deflated slowly (over 5-6 sec) so that there was adequate time for lung volume to essentially equilibriate with a continuous gradient of transpulmonary pressure. Maximal inflation was defined as lung volume at a transpulmonary pressure of 30 cm water. A schematic of the system is found in Figure 4(iii). One side of a differential pressure transducer was connected by a water filled tube to the tracheal cannula while the other side was attached to the esophageal cannula. Transpulmonary pressure was taken as the difference between esophageal pressure and tracheal pressure. The trachea was connected by a three-way valve to a 20-mL syringe. The other limb of this valve was connected to a solenoid which in turn was connected to a pressure flask maintained at -30 cm of water pressure.

The quasistatic maneuver was performed by injecting a volume of air equivalent to the inspiratory capacity, as defined earlier, slowly into the lungs, slowly withdrawing that volume of air over approximately 5-6 seconds, quickly switching the three-way valve to close off the syringe and connect the animal to the reservoir at a -30 cm water pressure. The body box was used as a plethysmograph during this maneuver. A 5-L flask was connected to the plethysmograph to prevent large pressure fluctuations. Lung volume changes were measured at increments in transpulmonary pressure of 5 cm water over inflation and deflation. Absolute lung volumes were calculated by combining FRC and the pressure-volume curves. Residual volume was defined as lung volume at a transpulmonary pressure of -30 cm water.

Organ Weight and Histopathology

The animals used in the pulmonary function tests were subsequently killed by aortic bleeding and several tissues were taken for weighing and histopathology. The right middle lung lobe was tied off at the bronchus, removed, and weighed immediately. It was then dried at 95°C for 2 days and reweighed for dry weight. The remainder of the lung and trachea were fixed for 24 hours under a constant tracheal pressure of 25 cm of buffered formalin. Liver, spleen, kidneys, adrenals and testes were removed for weighing prior to preservation in buffered formalin. The adrenals and the testes were weighed in pairs, while the kidneys were weighed individually. Other tissues removed and preserved in the buffered formalin were larynx, trachea, heart, aorta, tongue, palate, stomach, small intestine, large intestine, brain, nasal turbinates, spinal cord (lumbar region), sciatic nerve, sternum and eye. Other tissue available for examination included gross skin lesions and in many cases pancreas, thyroid, parathyroid, esophagus, pituitary, Harderian gland, teeth, ovary and uterus.

All tissues were routinely embedded in paraffin, sectioned and stained with hematoxylin and eosin. Cross-sections of the nasal turbinates were standardized using palate landmarks (12).

The tissue sections from all 128 rats were coded before histopathologic diagnosis. The pathologist diagnosed and interpreted the lesions without knowledge of the treatment groups to which the animals belonged or of the necropsy findings. After the histopathologic diagnoses were recorded, the pathologist was supplied with the necropsy findings and reviewed the microscopic findings for all those animals for which grossly visible lesions had been reported. The two data sets were determined to be compatible and no changes were made in the histopathologic diagnoses. Because foreign material had been discovered in the nasolacrimal ducts of some of the rats, the pathologist then reexamined the histologic sections of nasolacrimal ducts for all the rats. As the last step in the histopathologic analysis, the code was broken and the incidence of lesions was tabulated according to dosage of control groups.

Statistical Analysis

Unlike Phase II, which consists of incomplete block experiments, Phase III consists of complete factorial experiments. Thus, the statistical analysis is fairly straightforward. For each endpoint, after routine checking for outliers, an analysis of variance (13) was performed on the data. Since the data is balanced treatment comparisons are defined in the usual way, without ambiguity. Tables of means were computed, and standard errors were computed from the analysis-of-variance mean squared errors. If not quoted in the results, statistical significance will imply a significance level of 0.05. Further statistical details are included as results.

RESULTS

Aerosol Concentration

Continuous monitoring of the aerosol concentration was achieved for all exposures using infra-red backscatter detectors. Pad samples of chamber atmosphere were also taken at approximately the mid-point of exposure for gravimetric determination of chamber aerosol concentration. Table 1 provides a summary of target concentrations and the concentrations actually measured.

TABLE 1. MEAN AEROSOL CONCENTRATIONS (Mean ± SEM) AS DETERMINED GRAVIMETRICALLY AND BY INFRA-RED BACKSCATTER PROBES DURING A 15 MINUTE SAMPLING PERIOD AT THE CONCENTRATION PLATEAU AND AVERAGE CONCENTRATION DURING EACH ENTIRE FOUR HOUR EXPOSURE AS MEASURED BY INFRA-RED BACKSCATTER PROBES.

	Measured Concentrations (mg/L)		
	Gravimetric	Backscatter Probes	
Target Concentration		Over 15 min Interval	Over 4 hour Exposure
0.25 mg/L	0.35 ± 0.011	0.19 ± 0.004	0.17 ± 0.004
0.75 mg/L	0.88 ± 0.016	0.87 ± 0.021	0.87 ± 0.012
1.50 mg/L	1.71 ± 0.018	1.57 ± 0.022	1.58 ± 0.023

The gravimetric results show that the concentration was slightly above target whereas at the 0.25 mg/L the backscatter probes indicated that concentration was less than that targeted. It is believed that at extremely low concentrations the vapor phase represents a more significant percentage of the atmosphere and that during pad sampling this vapor recondenses within the matrix of the pad. To illustrate this a limited study on concentration of the vapor phase was carried out and was found to be around

13 percent in the range of 0.26 to 0.38 mg/L of diesel fuel aerosol and closer to 10 percent when aerosol concentration was increased to 1 mg/L. Aerosol concentrations overall were thus considered to be close enough to target concentration so that the study could be considered to have been carried out in accordance with experimental design. Furthermore standard errors are demonstrably small, indicating that variations between exposures were not large. These data also indicate the value of continuous monitoring for the duration of each exposure.

Particle size was measured for each exposure concentration with mass median aerodynamic diameter (MMAD) varying between 0.43 μm and 0.75 μm according to the aerosol concentration and the zone of the chamber from which the sample was drawn. It was found that particle size increased as chamber concentration increased (0.43 μm at 0.25 mg/L versus 0.57 μm at 1.5 mg/L) and that there was some particle growth as the aerosol passed from the top to bottom of the chamber with an increase of up to approximately 30 percent in MMAD. Standard geometric deviation (σg) was in the range of 1.4 to 1.7.

Mortality

Unlike the Phase 2 work which was a study using aerosol concentration-time combinations that were at the lower confidence bounds of 1 percent mortality, the concentrations chosen for this sub-chronic study were not expected to cause any mortality. During the entire 13 weeks of exposure and 8 week recovery period no deaths were observed.

Clinical Signs

During aerosol or sham exposures animals, that could be observed, remained relatively inactive throughout the exposure period. On removal from the chambers there were no overt clinical signs in the aerosol exposed animals that distinguished them from either the sham exposed or the vivarium control animals.

Body Weight

Mean cumulative body weight loss or gain for males and females are shown in Figures 5 and 6 respectively. Each individual animal served as its own control and the body weight recorded prior to treatment was subtracted from any recorded body weight to give overall weight loss or gain during the inclusive period. Actual body weights are shown in Tables 3A and 4A. It is obvious from these figures that there was a loss in weight as a result of exposure. The loss was most apparent over the first week and occurred in the sham exposed group although to a lesser extent than in the aerosol exposed groups. After the initial depression in body weight, the sham exposed controls appeared to grow normally since their cumulative weight gain curve parallels the vivarium controls for the remaining duration of the experiment.

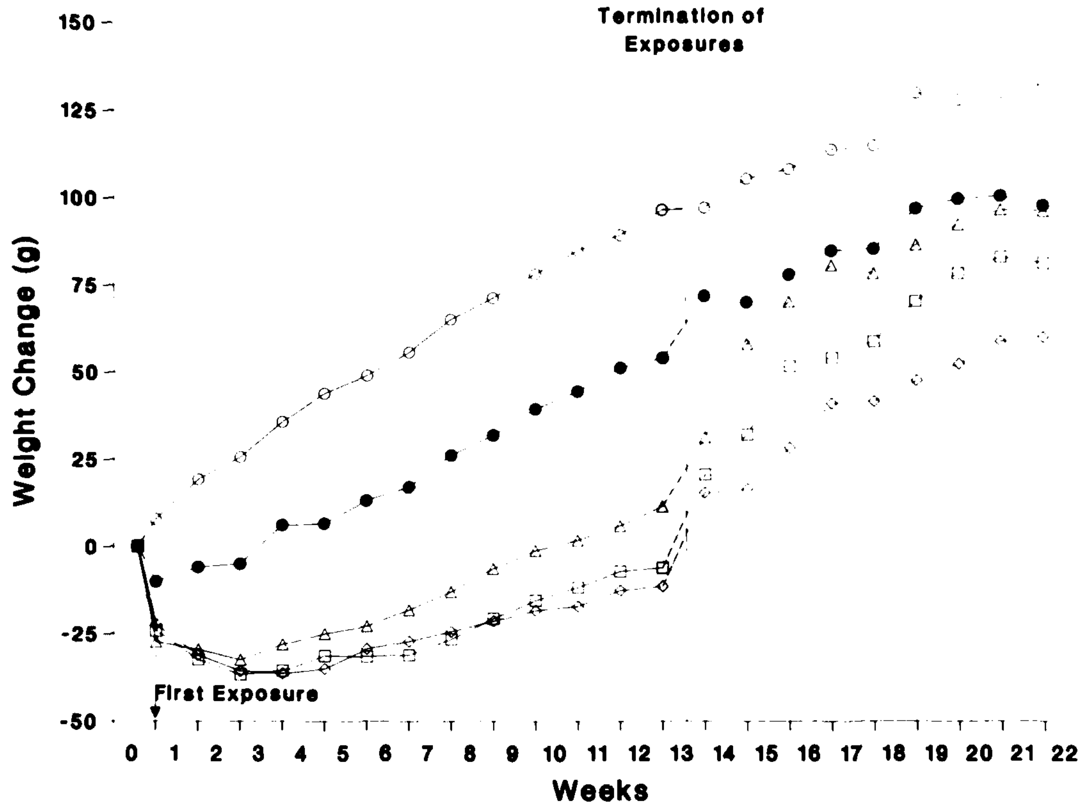

Figure 5

Cumulative weight change in male Sprague-Dawley rats exposed to diesel fuel aerosol. ○, Unloaded control; ●, sham exposed controls; △, 0.25 mg/L; ◇, 0.75 mg/L; □, 1.50 mg/L.

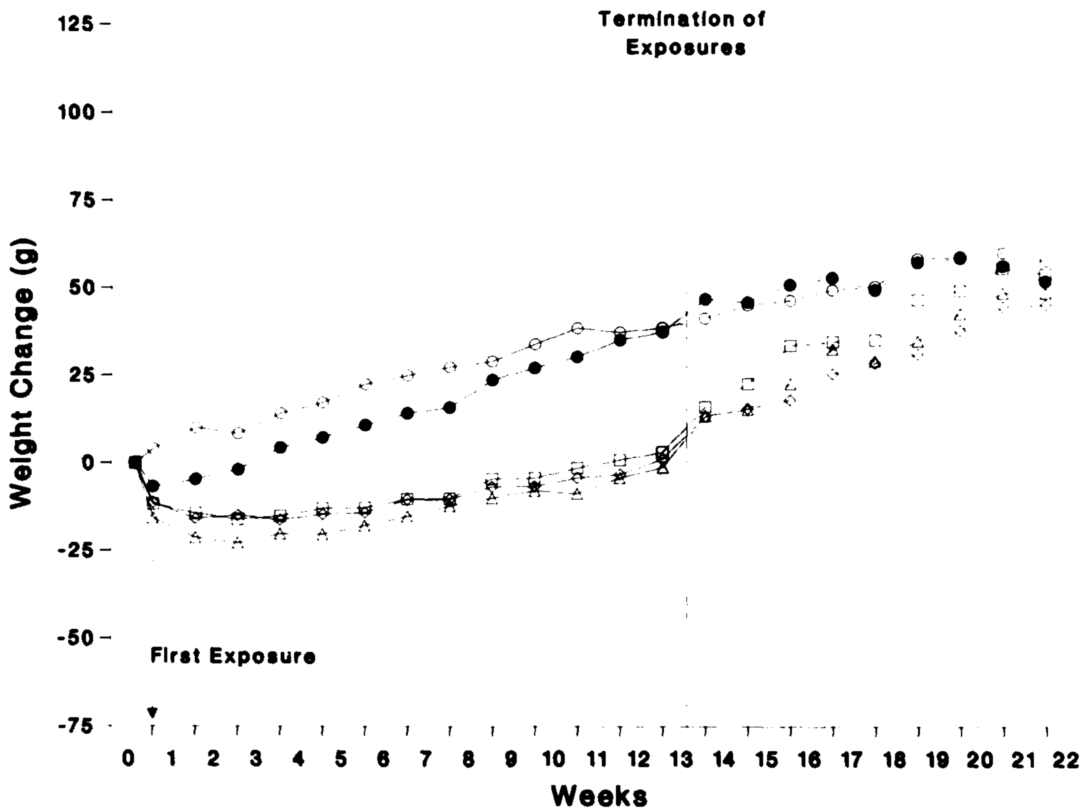

Figure 6

Cumulative weight change in female Sprague-Dawley rats exposed to diesel fuel aerosol. ○, Unloaded controls; ●, sham exposed controls; △, 0.25 mg/L; ◇, 0.75 mg/L; □, 1.50 mg/L.

Exposed animals, however, continued to lose weight until the beginning of the fourth week of exposure. After the fourth week all exposed animals started to gain weight and the males in the group exposed to 0.25 mg/L gained more rapidly than those in the other two (0.75 mg/L and 1.50 mg/L) groups. In females there was little gain in weight, following the initial depression, throughout the exposure period regardless of exposure concentration.

During the two month recovery period there were some important changes in weight gain. In females all exposed groups grew more rapidly, so that at the end of the recovery period these animals had, overall, gained as much weight as their sham exposed counterparts (Figure 6). On the other hand with the male rats, whilst weight gain was more rapid than during exposure, only the group exposed to 0.25 mg/L approached the overall weight gain posted for the sham exposed controls. The group exposed to 1.50 mg/L had the lowest overall weight gain.

The weight data were treated statistically by analysis of variance of the cumulative weight gains (or losses). Throughout the period of exposure weight gain was significantly lower ($p < 0.0001$) in the treated groups when compared with the sham exposed group, regardless of sex. In males, from the tenth week until the end of exposure, groups exposed to 0.75 mg/L and 1.50 mg/L were also significantly different ($p < 0.03$) from the group exposed to 0.25 mg/L. During the recovery period, the sham-exposed animals were statistically significantly higher than the treated groups ($p < 0.05$) except for the last two weeks in males and the last four weeks in females. The differences between the exposed groups were not statistically significant during the recovery period.

The observed changes in body weight indicate that the aerosol concentrations used could still be considered to be in the toxic range and that the "no observable effect level" (NOEL) has not been reached.

Food Consumption

Six male and six female rats from each group had their respective food consumptions recorded weekly throughout the exposure and recovery period. The data were examined and it was found that the effects of dose did not differ between the sexes. Thus, the conclusions are based on data from both sexes combined within each treatment group. The data for the individual sexes appear in Tables 5A and 6A, whilst Table 7A shows the data collapsed across sexes.

Throughout the study the group exposed to 0.25 mg/L did not differ significantly from the sham exposed group. With the exceptions of data points 7 and 10 (see Tables 5A - 7A), the groups exposed to 0.75 mg/L and 1.50 mg/L consumed significantly less ($p < 0.05$) than their sham exposed counterparts, from the fourth through the twelfth measurements. In some but not all cases a concentration-related effect may be observed. Prior to the fourth measurement and after the twelfth measurement there were no statistically significant differences amongst the groups, nor is there any evidence for trends indicating concentration related effects.

Breathing Frequency

Over the course of the experimental and recovery period the sham exposed male rats showed a steady decrease in breathing frequency (Table 8A). Similar decreases were observed in all males exposed to diesel fuel aerosol, and there were no statistically significant differences between exposed and control animals.

In the females the breathing frequencies at the different time points showed a more erratic pattern (Table 9A); however, there were no statistically significant differences between the exposed and control groups at any of the time points examined.

Changes in breathing frequency were calculated by subtracting the pre-exposure value for each individual animal from the breathing frequency for that same animal at the time point of interest. There were no statistically significant differences observed nor were there any evident trends relating to exposure concentration.

Overall it can be concluded that there are no chronic effects on breathing frequency resulting from exposure of rats to low diesel fuel aerosol concentrations.

Startle Reflex Assay

Data derived from the startle reflex assay included reaction time (msec from the start of the sound stimulus to the start of the response), peak time (msec from the start of the stimulus to maximal response) and the maximum force exerted (in g.wt and derived from the magnitude of the maximum response by using the calibration for the strain gauge). There were some statistically significant alterations observed in startle reflex in animals exposed to low concentrations of diesel fuel aerosol. As in the phase 2 study, pre-exposure values served as baseline data and were subtracted from observations made both during the exposure cycle and the recovery period to give a change in the effect being measured. In the first phase of the analysis, reaction times of less than 5 msec or greater than 40 msec were rejected as abnormal responses. The basis for these rejection criteria was that a response less than 5 msec or greater than 40 msec was most likely to be a spurious movement of the animal rather than a reaction to the startling stimulus. These criteria were established during phase 2 and result from studying over 2000 individual responses.

Out of a total of 3120 potential responses, in phase 3, only 21 responses (<0.7 percent) were rejected using the criteria described above. The data were then examined for effects of exposure on the number of responses. There were a total of 624 animal trials of which only 54 showed less than 5 responses. A breakdown of number of responses is given in Table 2.

TABLE 2. SUMMARY OF THE NUMBER OF RESPONSES IN THE STARTLE REFLEX ASSAY

Number of Responses	Animal Trials Included	Percentage of Total
5	570	91.35
4	42	6.73
3	9	1.44
2	1	0.16
1	2	0.32

There was no significant difference in number of responses between assays performed one day before an exposure (male and female) or immediately after an exposure (males only) to low concentrations of diesel fuel aerosol. During the recovery period there were also no observable differences in the number of responses.

Reaction time, peak time, and maximum force exerted (derived from peak height) (Figure 7) were analyzed by looking at changes from the respective pre-treatment values. The results are summarized in Tables 10A - 15A.

In male animals reaction time was significantly increased ($p < 0.05$) immediately after exposure to 1.50 mg/L at each time point studied, after the 1st and 14th exposure in animals exposed to 0.75 mg/L and after the 26th exposure in animals exposed to 0.25 mg/L. Although statistically significant differences were observed, their biological importance is in doubt, since the changes observed in reaction time were in the range of 1 to 2 msec. Previously, we have observed increases in reaction times of 17-20 msec (unpublished observations) when animals were exposed to 6 mg diesel aerosol/L for 2 hours. Before exposures 14 and 26, and during the recovery period, no males showed any significant evidence of residual effects as a result of exposure to diesel fuel aerosol; however, female rats exposed to 0.75 mg/L showed statistically significant differences prior to the 14th and 26th exposure. These longer reaction times are again considered to be of small consequence, since they represent increased reaction times of less than 2 msec compared with sham exposed controls.

Pretreatment values for the time to reach the maximum amplitude of the response (peak time) was statistically significantly higher in the group designated as sham exposed controls than in the other groups in the males (Table 12A). Since a similar effect was not observed in the females it can be stated that this difference was in all probability not due to any problem with the equipment but was a real effect. There is no explanation as to why this should occur since all animals at this stage were untreated

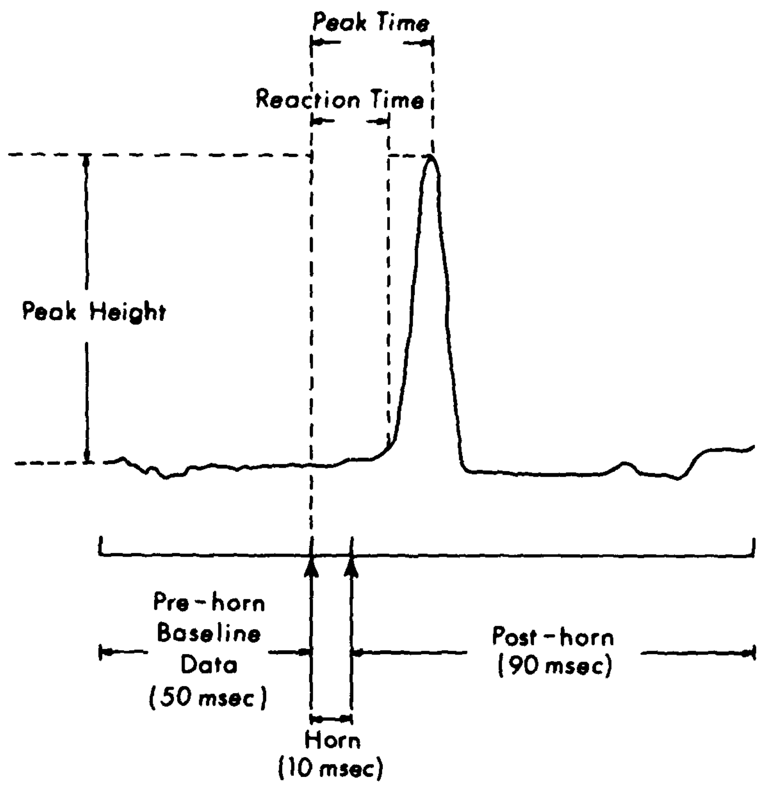

Figure 7

A typical signal obtained from the load cell when an animal is responding to the acoustic stimulus.

and in apparent good health. Furthermore, since the increased time to reach peak time in the control males is not reflected either in reaction time or the force exerted, it is indicating that the duration of response was being altered by approximately 2.5 msec without any corresponding alteration in the force which the animals are exerting on the strain gauge.

Since subsequent peak times are expressed as changes from the pretreatment values, if the sham exposed males retain this deviation then the use of differences negates it. Comparing the changes in reaction time and peak time after sham exposure shows that these animals responded in such a way that differences from pretreatment values for both sets of data remained within approximately 2 msec of each other, allowing us to conclude that the pretreatment values observed for peak time in the sham exposed animals was not of any great biological importance.

There were some statistically significant changes in peak time of males, particularly in the group exposed to 1.5 mg/L, where every value through to the time of sacrifice was different ($p < 0.05$) from the sham exposed controls. Groups exposed to 0.25 and 0.75 mg/L both showed statistically significant differences for males ($p < 0.05$) after the 14th and 26th exposures. Actual differences varied up to 5 msec and so it is believed that here some decrement in performance was observed. In females these differences were also observed in the 1.50 mg/L exposed group; however, statistical significance had been lost after 2 months of recovery.

Changes in peak height in most cases were not significantly altered by exposure to diesel fuel aerosol (Tables 14A and 15A).

Pulmonary Free Cells

Alveolar macrophages, lavaged from the lungs of diesel aerosol exposed male rats shortly (4 days) after the last exposure, were elevated in numbers over the sham exposed controls (Table 16A). Since the data were skewed, the least squares means were calculated on a square root transformation of the data (Table 17A). There was no concentration-related elevation in cell numbers, but the elevations observed for the 0.25 mg/L and 1.50 mg/L groups were statistically different when compared to sham exposed animals ($p < 0.05$). With the females the elevations in alveolar macrophages as a result of diesel fuel aerosol exposure were less obvious and not one of the exposed groups showed a significant elevation. After two months of recovery there were no significant differences between treated and control groups in either male or female animals.

Total lavaged cells (Table 18A) showed a similar pattern to that observed for the alveolar macrophages. However, when the number of cells other than alveolar macrophages were expressed by carrying out a square root transformation on the skewed data before calculating least squares means and levels of significance (Table 19A) there were no significant differences between the various groups in either the males or the females.

By collapsing the groups across sex and time, it is demonstrated (Table 3) that animals exposed to 0.25 mg/L and 1.50 mg/L have significantly elevated numbers of alveolar macrophages but numbers of cells other than alveolar macrophages are not affected by treatment.

TABLE 3. LEAST SQUARE MEANS OF THE SQUARE ROOT TRANSFORMATION OF ALVEOLAR MACROPHAGES (millions) AND OTHER CELLS (millions) LAVAGED FROM THE LUNGS VERSUS CONCENTRATION, SEX AND TIME POSTEXPOSURE

Concentration	Alveolar Macrophages	Other Cells
Control	1.49 ± 0.16	0.51 ± 0.20
0.25 mg/L	1.75 ± 0.17[a]	0.58 ± 0.21
0.75 mg/L	1.61 ± 0.19	0.28 ± 0.23
1.50 mg/L	1.77 ± 0.18[a]	0.45 ± 0.22

Sex	Alveolar Macrophages	Other Cells
M	1.37 ± 0.13	0.62 ± 0.15
F	1.95 ± 0.31	0.29 ± 0.37

Time Postexposure	Alveolar Macrophages	Other Cells
Immediately	1.77 ± 0.17	0.54 ± 0.21
After 2 mo.	1.55 ± 0.17[b]	0.37 ± 0.21[b]

a. Significantly different from control values ($p < 0.05$).

b. Significant difference between *immediately post treatment and two* months recovery ($p < 0.05$).

When the data are collapsed across group and time, no significant differences are observed between the sexes in cell numbers. Finally, collapsing the data across groups and sex, it can be seen that alveolar macrophages and other cell types are both elevated immediately post treatment, compared with values obtained after two months of recovery.

Pulmonary Function Tests

Results in this section are presented in the order in which the tests were carried out. Resistance was the first test and represents the resistance of the respiratory tract below the tracheal cannula. The results are summarized in Table 20A. No differences in resistance were observed that could be related to exposure, nor were there any sex related differences (Table 4); however, there was a statistically significant difference ($p < 0.0004$) between animals tested at the end of exposure and those tested after 2 months recovery.

TABLE 4. LEAST SQUARE MEANS OF LUNG RESISTANCE VERSUS CONCENTRATION, SEX AND TIME POSTEXPOSURE

Concentration	Resistance ($cm\ H_2O/mL/sec$)
Control	0.21 ± 0.03
0.25 mg/L	0.18 ± 0.03
0.75 mg/L	0.14 ± 0.03
1.50 mg/L	0.16 ± 0.03

Sex	Resistance ($cm\ H_2O/mL/sec$)
M	0.16 ± 0.02
F	0.19 ± 0.02

Time Postexposure	Resistance ($cm\ H_2O/mL/sec$)
Immediately	0.12 ± 0.02
After 2 mo.	0.22 ± 0.02[a]

a. Significant difference between immediately post treatment and two months recovery ($p < 0.0004$).

Multibreath nitrogen washout can be analyzed in a variety of different ways. Each method attempts to determine how efficiently the nitrogen present in the lungs is removed over a series of breaths of pure oxygen. One method is to express N_2 concentrations in the expired air at the end of exhalation in terms of breath number when tidal volume is assumed constant. The nitrogen concentration at the end of exhalation is taken as the best estimate of alveolar concentration. It was found in the phase 2 assays (4) that in order to compensate for changes in the resting volume of the lung (functional residual capacity or FRC) as a result of treatment it is necessary to calculate end-tidal N_2 concentration on the basis of the number of times that FRC was diluted by successive breaths. One analysis performed on the basis of cumulative dilutions of FRC was the simple linear regression of the log percent N_2 versus dilutions of FRC. This analysis assumes that the lungs washed out exponentially like a single compartment. The correlation coefficients of better than 0.98 attest to the validity of the one-compartment model. Thus the slope of the regression equation can be taken as an index of the rate of clearance of N_2 from the lung. Data are summarized in Table 21A. After two months of recovery, animals that had been exposed to 0.25 mg/L or 0.75 mg/L were significantly different from the control animals. This was true both in males and females, whereas immediately post treatment the only group that was significantly different ($p < 0.05$) from controls was the males exposed to 0.25 mg/L. When groups were collapsed across sex and time postexposure (Table 5), these same two exposure groups had significantly higher clearance rates than did the sham exposed controls, while the group exposed to 1.50 mg/L was in fact marginally lower than the controls.

TABLE 5. LEAST SQUARE MEANS OF NEGATIVE SLOPE OF THE LINEAR REGRESSION EQUATION FOR NITROGEN WASHOUT CURVES[a] VERSUS CONCENTRATION, SEX AND TIME POSTEXPOSURE

Concentration	Negative Slope
Control	0.12 ± 0.003
0.25 mg/L	0.14 ± 0.003[b]
0.75 mg/L	0.13 ± 0.003[b]
1.50 mg/L	0.11 ± 0.003

Sex	Negative Slope
M	0.159 ± 0.002
F	0.093 ± 0.002[c]

Time Postexposure	Negative Slope
Immediately	0.125 ± 0.002
After 2 mo.	0.126 ± 0.002

a. y = log (percent N_2).
x = Cumulative dilutions of FRC.

b. Significantly different from controls ($p < 0.002$).

c. Females significantly different from males ($p < 0.0001$).

There is no explanation readily available for this biphasic phenomenon, however, we had made similar observations with some of the pulmonary function tests in the phase 2 work.

By collapsing data across exposure group and time postexposure it was possible to show that nitrogen washout was significantly slower in females. This is not a new finding but rather reinforces information that is already available.

The number of dilutions to reach nitrogen concentrations of 10 percent and 5 percent at the end of exhalation were calculated by extrapolation of data points on either side of these nitrogen concentrations for each animal. Data is summarized in Tables 22A and 23A.

An estimate of the efficiency of gas exchange between the lungs and the circulating blood was obtained by measuring the rate of diffusion of carbon monoxide from the lung in a single breath maneuver. Results, expressed as mL CO transferred/min/mmHg, are summarized in Table 24A. Two groups showed statistically significant differences from the sham exposed controls, but the biological significance of these statistical differences is probably of no consequence. A true picture of how sex, exposure concentration and time postexposure affect diffusing capacity can not be obtained since it was technically not possible to obtain results for this test for one of the exposure groups immediately post treatment.

A maximal forced exhalation maneuver was carried out to test for functional obstruction of the airways. In this test animals had their lungs inflated to TLC using a positive pressure of 30 cm H_2O and then rapidly deflated by connecting the lungs to a flask held at -30 cm H_2O. [In this way the respiratory system limited the maximum flow at any given lung volume.] Expiratory flow was analyzed in terms of peak flow, flow at 50 percent of vital capacity and flow at 25 percent of vital capacity. Original peak flows are shown as mean and standard error of the mean in Table 25A. No significant differences were observed. Collapsing the data across sex, time and group shows (Table 6) that exposure concentration has no effect on peak flow but that the females, as expected, have statistically significant lower peak flows than the males. Also it may be seen that time postexposure has an effect on peak flow but this is probably a function of age rather than a treatment related effect.

TABLE 6. LEAST SQUARE MEANS OF PEAK FLOW VERSUS CONCENTRATION, SEX AND TIME POSTEXPOSURE

Concentration	Peak Flow (mL/sec)
Control	41.7 ± 1.2
0.25 mg/L	38.6 ± 1.2
0.75 mg/L	41.1 ± 1.1
1.50 mg/L	41.8 ± 1.2

Sex	Peak Flow (mL/sec)	Time Postexposure	Peak Flow (mL/sec)
M	43.29 ± 0.81	Immediately	44.14 ± 0.81
F	38.39 ± 0.84[a]	After 2 mo.	37.54 ± 0.84[b]

a. Females significantly different from males ($p < 0.0001$).

b. Significant difference between immediately post treatment and two months recovery ($p < 0.0001$).

Flows at 50 percent of vital capacity and 25 percent of vital capacity showed identical patterns, with significant differences between the sexes and significant differences between the values obtained immediately post treatment and those obtained following two months of recovery.

Since maximal flow is related to vital capacity it was of interest to learn whether there were any observable effects when ratios of flow to vital capacity were calculated. As may be seen in Table 26A, there are no significant differences within sex and treatment times but there are some apparent differences between the sexes and between animals tested immediately post-treatment and after two months recovery. When groups are collapsed across dose and time or dose and sex, statistically significant differences are observed. In the first case a difference between sexes might be expected, while in the second case changes are most probably age related since there was no evidence of any pulmonary infection being endemic within the colony.

Two tests were carried out to study the effects of the aerosol on different lung volumes. Functional residual capacity (FRC) was obtained using the Boyle's Law method. Absolute lung volumes derived by combining the the continuum of volumes obtained from the quasistatic pressure-volume curves with the calculated values of FRC included total lung capacity (TLC), vital capacity (VC), inspiratory capacity (IC) and residual volume (RV). Changes in these lung volumes can be used as an indicator of changes in the structural and/or elastic properties of the lung.

Values for the different lung volumes mentioned above are given in Tables 27A - 31A. When TLC was analyzed by the method of least square means it was found that animals exposed to 1.50 mg/L had significantly lower values than the sham exposed controls. Similarly females had statistically significantly lower TLC than males but time postexposure had no influence on TLC. These results are summarized in Table 7.

TABLE 7. LEAST SQUARE MEANS OF TOTAL LUNG CAPACITY (TLC) VERSUS CONCENTRATION, SEX AND TIME POSTEXPOSURE

Concentration	TLC (mL)
Control	17.60 ± 0.43
0.25 mg/L	17.41 ± 0.41
0.75 mg/L	16.86 ± 0.40
1.50 mg/L	16.37 ± 0.42[a]

Sex	TLC (mL)
M	20.30 ± 0.30
F	13.82 ± 0.29[b]

Time Postexposure	TLC (mL)
Immediately	16.81 ± 0.24
After 2 months	17.30 ± 0.30

a. Significantly different from controls ($p < 0.05$).

b. Females are significantly different from males ($p < 0.0001$).

As expected, the males, because of their larger body size, had greater TLC's; however, as in the phase 2 study (4) when TLC was referenced to body weight, it was the females that had the significantly greater ($p < 0.0001$) lung volumes in proportion to their body weight (48.75 ± 0.09 μL/g vs 38.83 ± 0.09 μL/g). A comparison between the different times postexposure showed that, when referenced to body weight, TLC was significantly higher (0.02) in the animals tested immediately postexposure than in those allowed to recover for two months. This difference is probably accounted for by an increase in body fat in the older animals.

Vital capacity was not significantly affected by exposure or by time postexposure but, as might be expected, there was a significant sex difference (Table 8).

TABLE 8. LEAST SQUARE MEANS OF VITAL CAPACITY (VC) VERSUS CONCENTRATION, SEX AND TIME POSTEXPOSURE

Concentration	VC (mL)
Control	15.55 ± 0.40
0.25 mg/L	15.30 ± 0.38
0.75 mg/L	14.96 ± 0.37
1.50 mg/L	14.58 ± 0.39

Sex	VC (mL)	Time Postexposure	VC (mL)
M	17.80 ± 0.27	Immediately	14.78 ± 0.17
F	12.40 ± 0.28[a]	After 2 months	15.41 ± 0.27

a. Females are significantly different from males ($p < 0.0001$).

When VC was referenced to body weight, as with TLC, the females showed proportionally greater volumes for their body weight than did males (43.70 ± 0.08 μL/g vs 34.03 ± 0.08 μL/g). When comparisons were made between the vital capacities referenced to body weight at different times (i.e., immediately post-treatment vs two months recovery), it was found that again there was a statistically significant difference ($p < 0.05$) between the two time periods. As with TLC, this can probably be accounted for by increased body fat in the older animals without the concomitant increase in overall body size.

Inspiratory capacity paralleled VC with a significant sex difference ($p < 0.0001$), but no apparent effect on exposure concentration or time postexposure (Table 9).

TABLE 9. LEAST SQUARE MEANS OF INSPIRATORY CAPACITY (IC) VERSUS CONCENTRATION, SEX AND TIME POSTEXPOSURE

Concentration	IC (mL)
Control	14.56 ± 0.36
0.25 mg/L	14.08 ± 0.34
0.75 mg/L	13.83 ± 0.34
1.50 mg/L	13.60 ± 0.35

Sex	IC (mL)
M	16.49 ± 0.24
F	11.54 ± 0.25[a]

Time Postexposure	IC (mL)
Immediately	13.75 ± 0.24
After 2 months	14.29 ± 0.25

a. Females are significantly different from males ($p < 0.0001$).

Exposure of the animals to low concentrations of diesel fuel aerosol had no affect on FRC (Table 10). This is contrary to what had been observed in the phase 2 study (3) where exposure to higher aerosol concentrations had caused an increase of up to 15 percent in FRC. As with the other lung volumes discussed thus far, a sex difference was observed.

TABLE 10. LEAST SQUARE MEANS OF FUNCTIONAL RESIDUAL CAPACITY (FRC) VERSUS CONCENTRATION, SEX AND TIME POSTEXPOSURE

Concentration	FRC (mL)
Control	3.04 ± 0.11
0.25 mg/L	3.33 ± 0.10
0.75 mg/L	3.03 ± 0.10
1.50 mg/L	2.77 ± 0.10

Sex	FRC (mL)
M	3.80 ± 0.07
F	2.28 ± 0.07[a]

Time Postexposure	FRC (mL)
Immediately	3.07 ± 0.07
After 2 months	3.02 ± 0.07

a. Females significantly different from males ($p < 0.0001$).

Residual volume followed the same pattern as FRC; however, at an exposure concentration of 1.50 mg/L, RV was significantly lower than in the control animals (Table 11). Residual volume was also influenced by sex with the females statistically significantly lower ($p < 0.0001$).

TABLE 11. LEAST SQUARE MEANS OF RESIDUAL VOLUME (RV) VERSUS CONCENTRATION, SEX AND TIME POSTEXPOSURE

Concentration	RV (mL)
Control	2.05 ± 0.08
0.25 mg/L	2.11 ± 0.08
0.75 mg/L	1.90 ± 0.08
1.50 mg/L	1.79 ± 0.08[a]

Sex	RV (mL)	Time Postexposure	RV (mL)
M	2.50 ± 0.06	Immediately	2.04 ± 0.06
F	1.43 ± 0.06[b]	After 2 months	1.89 ± 0.06

a. Significantly different from controls ($p < 0.03$).

b. Females are significantly different from males ($p < 0.0001$).

Lung volumes discussed to this point have all been derived using FRC and the quasistatic P-V curves to give absolute lung volumes. An alternative method of examining pressure-volume curves is by comparing lung compliance. Specific compliance is defined as the slope of the P-V curve over a specified range of transpulmonary pressure divided by the actual lung volume at the midpoint of the selected range of transpulmonary pressures. As might be expected, specific compliance varies in different regions of the same P-V curve; thus a specific range is used to define the area of the curve being examined. The values of specific compliance given in Table 32A were derived by examining the slope for transpulmonary pressures from 0 to 10 cm H_2O and dividing by the lung volume at 5 cm H_2O.

A least squares means analysis of specific compliance did not show any significant effects as a result of exposure or the time after exposure that the animals were tested, but there was a significant sex effect with females showing significantly greater specific compliance than males (Table 12).

TABLE 12. LEAST SQUARE MEANS OF SPECIFIC COMPLIANCE (C_{sp}) VERSUS CONCENTRATION, SEX AND TIME POSTEXPOSURE

Concentration	C_{sp} (mL/cm H_2O/mL)
Control	1.24 ± 0.04
0.25 mg/L	1.23 ± 0.04
0.75 mg/L	1.19 ± 0.04
1.50 mg/L	1.26 ± 0.04

Sex	C_{sp}
M	1.18 ± 0.03
F	1.24 ± 0.03[a]

Time Postexposure	C_{sp}
Immediately	1.27 ± 0.03
After 2 months	1.19 ± 0.03

a. Females are significantly different from males ($p < 0.0001$).

Organ Weights

When the animals used in the pulmonary function tests were sacrificed, a full necropsy was carried out on each animal. Wet weights of the liver, kidneys (individually), spleen, adrenals (both together), testes (both together) and the right middle lobe of the lung were obtained. As the weights of body organs are expected to vary on the average with body weight, and since overall weight changes associated with treatment have been determined, it is important in assessing the effect of treatment on organ weights to adjust for body weight. This was done by including body weights as covariates in the linear model relating organ weight to the various factors (i.e., concentration, sex, time postexposure, etc.) (13). Separate terms were used for males and females. Under this model the effect of body weight on organ weight can be estimated and thus eliminated as a source of bias in estimating the effect of other factors. Indeed we can estimate the results of a hypothetical experiment in which the body weights at the time of sacrifice are for every male, the overall mean for males, and for every female, the overall mean for females. Mean weights adjusted for body weight, are recorded in Tables 33A to 38A.

After adjusting for body weight there were no significant differences in liver weight between males and females. Exposure to diesel fuel caused an increase in liver weight (Table 13) but the increase was not demonstrated to be concentration dependent since exposures of 0.25 mg/L and 1.50 mg/L caused significant ($p < 0.05$) increases in weight but a concentration of 0.75 mg/L did not cause any marked change.

TABLE 13. THE EFFECTS OF AEROSOL CONCENTRATION ON LEAST SQUARE MEANS OF LIVER WEIGHT ADJUSTED FOR BODY WEIGHT

Concentration	Liver Weight (g)
Control	10.41 ± 0.39
0.25 mg/L	11.24 ± 0.42[a]
0.75 mg/L	10.59 ± 0.45
1.50 mg/L	11.80 ± 0.44[a]

a. Significantly different from controls ($p < 0.02$).

In the phase 2 study we had observed that liver weight decreased with exposure to the diesel fuel aerosol while in this study we observed increases in liver weight.

Even after kidney weights were adjusted for body weight, males had significantly heavier kidneys than females ($p < 0.0001$), however, there were no statistically significant changes in kidney weight as a result of exposure to diesel fuel nor were there any effects related to time postexposure. On the other hand there were no sex or exposure related effects on spleen weight; however, spleens from animals immediately post-treatment were statistically significantly ($p < 0.002$) heavier (0.63 ± 0.03 vs 0.57 ± 0.03) than after two months recovery.

There were some statistically significant changes in adrenal weight as evidenced in Table 14. As with the liver, exposure to diesel fuel aerosol did cause an increase in adrenal weight with significant differences at concentrations of 0.25 mg aerosol/L and 1.50 mg aerosol/L. Adrenals were heavier in the females than in the males and also heavier immediately postexposure than after two months recovery.

TABLE 14. LEAST SQUARE MEANS OF ADRENAL WEIGHTS (mg), ADJUSTED FOR BODY WEIGHT, VERSUS CONCENTRATION, SEX AND TIME POSTEXPOSURE

Concentration	Adrenal Weight (mg)
Control	68.6 ± 0.5
0.25 mg/L	77.2 ± 0.6[a]
0.75 mg/L	71.6 ± 0.6
1.50 mg/L	79.3 ± 0.6[a]

Sex	Adrenal Weight (mg)	Time Postexposure	Adrenal Weight (mg)
M	51.9 ± 0.5	Immediately	88.4 ± 0.6
F	96.4 ± 0.9[b]	After 2 months	60.0 ± 0.5[c]

a. Significantly different from controls ($p < 0.05$).

b. Females are significantly different from males ($p < 0.0001$).

c. Significantly different from values immediately post-treatment ($p < 0.0001$).

There were no significant changes in the weight of the testes as a result of exposure, but at two months postexposure the testes were smaller than immediately post-treatment ($p = 0.004$).

The wet weight of the right middle lobe of the lung, adjusted for body weight, increased with increasing diesel fuel aerosol concentrations in both sexes (Table 38A). When groups were collapsed across sex and time post-treatment there is a clear concentration effect, although only the value for 1.50 mg/L is significantly different from controls (Table 15). When comparisons are made on the basis of time postexposure the values after two months recovery are significantly lower ($p < 0.0001$) than those immediately after exposure. Not surprisingly, there were no differences in wet weight between the sexes.

Although increases in wet weight were observed as a result of exposure, the ratio of wet weight to dry weight immediately after treatment did not change significantly (Table 39A). When wet weight increases as a result of exposure without a corresponding change in wet/dry ratio, the change results predominantly from increases in cellularity rather than development of edema. After a two month recovery period, animals that had been exposed to 1.5 mg/L showed a significant ($p < 0.0001$) increase in the wet/dry ratio (Table 16). This increase results from a significant drop in dry weight.

TABLE 15. LEAST SQUARE MEANS OF WET WEIGHT (mg) OF RIGHT MIDDLE LOBE OF LUNG (RML), ADJUSTED FOR BODY WEIGHT, VERSUS CONCENTRATION, SEX AND TIME POSTEXPOSURE

Concentration	Wet Weight RML (mg)
Control	153 ± 6
0.25 mg/L	157 ± 6
0.75 mg/L	160 ± 7
1.50 mg/L	172 ± 6[a]

Sex	Wet Weight RML (mg)
M	160 ± 5
F	161 ± 10

Time Postexposure	Wet Weight RML (mg)
Immediately	171 ± 6
After 2 months	150 ± 5[b]

a. Significantly different from controls ($p < 0.05$).

b. Significantly different from values immediately post-treatment ($p < 0.0001$).

TABLE 16. LEAST SQUARE MEANS OF WET/DRY RATIO OF RIGHT MIDDLE LOBE OF LUNG (RML), ADJUSTED FOR BODY WEIGHT, VERSUS CONCENTRATION, SEX AND TIME POSTEXPOSURE

Concentration	Wet/Dry Ratio
Control	5.30 ± 0.13
0.25 mg/L	5.21 ± 0.15
0.75 mg/L	5.21 ± 0.16
1.50 mg/L	6.36 ± 0.15[a]

Sex	Wet/Dry Ratio
M	5.55 ± 0.12
F	5.49 ± 0.23

Time Postexposure	Wet/Dry Ratio
Immediately	5.43 ± 0.15
After 2 months	5.62 ± 0.13[b]

a. Significantly different from controls ($p < 0.0001$).

b. Significantly different from values immediately post-treatment ($p < 0.04$).

Clinical Chemistry

At time of sacrifice blood samples were taken from six animals per sex per exposure concentration. The whole blood was used immediately for routine hematology (RBC, WBC and hematocrit) whilst the serum was frozen and sent to the Clinical Chemistry Department within Oak Ridge National Laboratory. Assays were carried out within 4 days of sacrifice of the animals.

There were no biologically significant changes in the clinical chemistry parameters which could be related to diesel fuel exposure, thus only least square means for sham exposed animals collapsed across time post-treatment are shown (Table 40A) to illustrate the type of data obtained. In females, differences from controls that were statistically significant were observed for LDH, potassium, cholesterol, uric acid and creatinine. With the exception of potassium and uric acid these differences all occurred in animals exposed to 1.5 mg/L and immediately post-treatment. For potassium, significant differences were observed for aerosol concentrations of both 0.25 mg/L and 1.5 mg/L immediately post-treatment whilst for uric acid these same two exposure concentrations showed significant differences after two months recovery. The only statistically significant change observed in males was an elevated LDH in the group exposed to 1.5 mg aerosol/L after two months recovery.

Results from the counts of RBC and WBC and the measurement of hematocrit showed that there were no significant biological effects occurring as a result of exposing rats to low concentrations of diesel fuel aerosol. Least square means of these parameters in sham exposed animals (collapsed across time post-treatment) are shown in Table 41A.

Histopathology

At time of sacrifice the animals and individual organs were carefully examined by a pathologist for evidence of macroscopic lesions. Of the animals sacrificed immediately after treatment, no macroscopic lesions were recorded for the control group or the group that had been exposed to 1.5 mg/L. Amongst animals exposed to 0.25 mg/L, 2 males had unilateral enlargement of the renal pelvis (UA-CR02M and UA-CR08M) and one male (UA-CR07M) had calculi in the renal pelvis and also small sand-like calculi in the bladder. Of the animals exposed to 0.75 mg/L two males (TA-CR03M and TA-CR04M) had a preputial gland abscess. One of them (TA-CR04M) also had ascending pyelonephritis in both kidneys and pus in the urine.

After two months of recovery, one female in the control group (SA-CR19F) had a small cortical cyst on one kidney and one female (SA-CR16F) had calculi in the urinary bladder. Amongst the treated groups, one female in the group exposed to 0.75 mg/L (TA-CR20F) had a small nodule in the mammary tissue which was subsequently diagnosed as a myxoma. No other gross lesions were recorded.

The results of the histopathological evaluation are summarized in Table 42A. For completeness, the summary table contains all diagnoses including those that affect single animals. Two analytical approaches were used. One was to search for lesions that might only occur in exposed animals and would also be concentration related. This would imply a cause and effect relationship. The other approach was to look, in exposed animals, for increased frequencies of lesions that are expected to occur spontaneously in rats of this strain and age. By using this latter analytical approach it was hoped to learn whether exposure to diesel fuel aerosol either exacerbated or accelerated the onset of naturally occurring lesions.

Since the primary route of exposure was by inhalation, special attention was directed toward the respiratory tract. No significant lesions were found in the upper respiratory tract (one control animal and one animal exposed to 1.5 mg/L had small amounts of purulent exudate in the nasal cavity) but tiny lesions were found in the lungs of a few rats in both control groups and in five of the six exposed groups. The pulmonary lesions were found in up to 4 rats per group (0 to 25 percent) in the peripheral alveolar portions of the lung with no particular relationship to bronchioles or large vessels. Typically, one or two foci, 100 to 200 μm in diameter, were found in each affected rat. Each focus consisted of a loose collection of foamy macrophages in a cluster of adjacent alveoli, sometimes with focal thickening of the associated alveolar walls due to mild epithelial hyperplasia. In eight of the sixteen rats with pulmonary lesions some macrophages contained greenish granular particulate material. Because the lesions are not considered to be related to the experimental procedure, no attempts were made to identify the foreign material. It should be emphasized that the pulmonary lesions were few, tiny, low in incidence, and found with comparable frequency in control and dosed rats. They are considered of no consequence and probably represent clearance of environmental particles, possibly of food origin. Crystalline and particulate phagocytized debris was found in the nasolacrimal ducts of 7/32 (21.9%) control and 19/96 (19.8%) dosed rats, without evidence of inflammation, hyperplasia or metaplasia of the ducts. This finding was considered of no importance to the study. It was concluded by the pathologist that no lesion in the respiratory tract could be related to the experimental procedure.

Several types of lesions expected in rats of this strain and age were found in the kidneys, adrenals and hearts. All the rats had varying degrees of glomerulosclerosis characterized by thickened basement membranes, occasional adhesions of glomerular loops to Bowman's membrane and occasional glomerular atrophy. In exposed animals permitted a recovery period after cessation of exposure and in their corresponding controls, the renal lesions were more severe in about half the rats, with proteinic tubular cast formation, focal interstitial infiltration of lymphoid cells and focal tubular regeneration. The increased severity of renal lesions in these groups can be attributed to the age of these rats at the time of death.

Small adrenal cortical adenomas were found in the longer-lived groups of rats (2/16 controls, 3/16 0.25 mg/L, 3/16 0.75 mg/L and 0/16 1.50 mg/L) but not in those sacrificed immediately after the end of the exposure period. One rat in the 0.25 mg/L group had a cortical adenoma in each adrenal. Separate analysis, taking into account the bilaterality of adrenal glands and slight differences in group sizes due to the amount of tissue available for evaluation revealed no differences between exposed and control groups.

In the hearts of one to four rats in each group, including controls, were tiny foci of degeneration of single cardiac fibers with associated lymphomonocytic inflammatory infiltrates. These lesions of unknown origin are commonly found scattered widely through the myocardium of Sprague-Dawley rats and are considered incidental and unrelated to the experimental procedure. One other finding was intestinal nematodiasis (pinworm) without enteritis in 7/32 (21.9%) control and 13/96 (13.5%) dosed rats. No other lesions occurred with sufficient frequency to deserve mention. Two incidental disorders of growth were found, a focus of adenomatous hyperplasia of the renal cortex in a control rat and a subcutaneous myxoma in the mammary area of in a rat exposed to 0.75 mg/L; neither is of biological or statistical significance. The myxoma, a discrete 2 mm diameter nodule, may represent an early fibroadenoma but it contained no glandular elements.

The conclusions of the histopathologic analysis are that no new lesions were found that could be attributed to inhalation of the diesel fuel aerosol and that the incidence and severity of naturally occurring lesions were not changed by administration of the test material.

DISCUSSION

This report is the third and final report in a series of studies designed to examine the potential health hazard associated with exposure to an aerosol generated from diesel fuel #2. In previous reports the effects of exposing rats once to high concentrations of the aerosol and exposing them a total of 9 times to different combinations of exposure time, duration of exposure and frequency of exposure have been discussed. In the series of single acute exposures it was observed that mortality was highly related to the Ct product (1). Thus this same expression of exposure 'dose' was used in the planning of the exposure regime for the repeat exposures. In Phase 2 it was found, in fact, that frequency of exposure was the dominant factor in determining the extent of the observed toxicity rather than either aerosol concentration or duration of exposure. The principle aim of the final series of experiments was to determine whether multiple exposures to low aerosol concentrations, that would not be considered acutely toxic, would cause any evidence of toxicity as a result of the multiple exposures.

In the exposures carried out under the aegis of Phase 2, the lowest aerosol concentration used was 1.33 mg/L with a corresponding exposure duration of 6 hours. In the study reported here 1.5 mg/L was selected as the maximum concentration to be used but the exposure time for all exposures was fixed at 4 hours. Thus while the highest concentration used in Phase 3 exceeded the lowest concentration used in the previous series of experiments if daily 'dose' was expressed in terms of the Ct product then animals were only exposed to a 'dose' that was 75 percent of the lowest one used in the Phase 2 studies. Other exposure concentrations selected were 50 percent of the maximum (0.75 mg/L) and the lowest concentration that could be consistently generated and which turned out to be 0.25 mg/L. The selection of the four hour exposure period was made using two criteria - an exposure time that was midway between the two exposure periods in Phase 2 and a time period that also represents half of a normal working day.

During the course of the exposures there were no visual signs of progressive systemic toxicity. However, changes in body weight during the course of the exposure clearly demonstrated that even at the lowest of the three concentrations some systemic toxicity was occurring. This toxicity was evidently reversible, since immediately after exposures were completed body weight of the aerosol exposed animals at all dose levels increased at a rate that was more rapid than that observed in the sham exposed control animals. By the end of a two month recovery period, animals that had been exposed to the diesel fuel aerosol were approaching the overall body weight gains of the sham exposed controls and, in the case of the females, the vivarium controls too. In the male animals there remained statistically significant difference between the vivarium and sham exposed controls. The exposed groups had lower weight gains than the sham exposed controls and there were statistically significant differences separating these groups from both control groups. Although during the exposure period there were significant differences between the sham exposed controls and the aerosol exposed groups in terms of absolute body weight the differences barely exceeded 10 percent of the sham exposed values. Thus if the definition of maximum tolerated dose (MTD) as defined in Phase 2 (4) (a 'dose' which produces greater than 10 percent depression in body weight) is used then it would appear that, for the exposure regime utilized in these experiments, MTD had been reached. It is important to realize that all animals used in this study were past the stage of rapid growth which occurs during the first weeks post-weaning and could be considered healthy young adult animals.

There were no permanent changes in startle reflex during the course of the exposures, clearly demonstrating that, at aerosol concentration levels used in this series of experiments, no cumulative CNS toxicity was occurring as measured by this endpoint. However, acute effects of the diesel fuel aerosol were noted in the males tested immediately postexposure. From the results obtained, it would appear that animals exposed to 1.5 mg/L showed the greatest change, while the lower concentration groups demonstrated less significant alterations. It must be stressed, however, that none of the changes in the startle response were as severe as those seen in some acute studies using aerosol concentrations that ranged from 0.5 to 6 mg/L (14).

Breathing frequency in all the male groups did appear to become slower during the course of the exposure and recovery periods but, since the control and diesel fuel aerosol exposed groups were almost identically affected, it is evident that the aerosol caused no cumulative effects. Results from the female groups were more erratic, but the overall conclusion that there were no diesel fuel aerosol related effects is still applicable.

Effects on the respiratory system were, as might be expected, not as marked during the Phase 3 study as those observed in the Phase 2 study. Slight increases in lavaged alevolar macrophages were observed in exposed animals immediately postexposure; however, there did not appear to be any concentration related effects in either male or female. After a two month recovery period, lavaged alveolar macrophage numbers were consistent within each sex regardless of treatment. Other cells lavaged from the lung showed a slight depression in females exposed to either 0.75 or 1.5 mg diesel fuel aerosol/L immediately postexposure, however, this depression was not statistically significant. Examination of histological sections confirmed that there was no important influx of any type of free cell.

Since aerosol particle size was less than 1 μm (MMAD) no histological changes in the airways were either expected or observed. Resistance, one of the primary pulmonary function tests that would demonstrate changes in airway pathology was not changed immediately post-treatment; however, in males, there appeared to be a concentration related effect after the two month recovery period. Highest resistance was observed in the sham exposed animals. This apparent increase in resistance in control animals may be an artifact totally unrelated to the experimental procedures since there was no evidence of infection in any of the animals used in the pulmonary function tests. Overall, it is found that when groups are collapsed across sex and aerosol concentration resistance is statistically significantly higher in all animals allowed to recover for two months. This increase in all likelyhood is related to animal age or some slight change in the pulmonary function apparatus rather than important change in airway physiology as a result of exposure to diesel fuel aerosol.

There appears to be a trend toward smaller lung volumes (TLC, VC, IC, FRC and RV) in animals exposed to diesel fuel aerosol. There is no evidence of any histopathological lesions that would account for this trend, and body weight differences between the groups were probably sufficient to account for this variation. Gas exchange, as evidenced by both the nitrogen washout assay and the carbon monoxide diffusing capacity, is not affected in any manner that relates to concentrations of aerosol to which the animals have been exposed, and this clearly demonstrates that there are no induced changes to gas exchange mechanisms as a result of exposure to low levels of diesel fuel aerosol. Dynamic lung function tests such as forced expiratory flow and specific compliance clearly show that exposure to low diesel fuel aerosol concentrations is not affecting the elastic properties of the lung in any way.

The minor functional and volume changes in the lung are not supported by the histopathology and thus are not likely to represent irreversible or clinically significant events. Furthermore the magnitude of the physiological changes do not warrent any morphometric studies.

Amongst the tissue weights the changes observed in the adrenals, when adjusted for body weight, stand out above all others. There is a very clear difference between the sexes, with females having adrenals approximately double that of males when data is collapsed across aerosol concentration and time. This is not an unexpected observation since in general it is known that females do have larger adrenals when body weight is factored in. Collapsing data across sex and time postexposure, while not demonstrating a clear cut aerosol concentration/response certainly gives evidence that aerosol exposure does increase adrenal weight. This weight increase seems to largely disappear during the recovery period which suggests that a stress factor may be implicated in these findings. It is not possible to evaluate the adrenals histologically to determine whether there were any changes in the cortical/medullary ratio since the way in which the tissues were cut made it impossible to ensure a standard section across the largest diameter.

The other tissue to show significant weight gains was the lung (based on the data obtained from the right middle lobe). Immediately postexposure the weight of RML (adjusted for body weight) of both males and females exposed to 1.5 mg diesel fuel aerosol/L were significantly elevated over those of the corresponding sham exposed control animals whilst after the two month recovery period these differences were no longer in evidence. When the wet/dry ratio of RML adjusted for body weight are examined the converse is found with both males and females in the 1.5 mg diesel fuel aerosol/L group being significantly elevated over sham exposed controls after the two month recovery period. It would appear that dry weight is the major factor in this phenomenon, being much lower than in the other groups. The first explanation for this unusual observation might be that these lungs were dried more thoroughly than the other groups; however, all parameters were carefully controlled during the drying to constant weight. Thus it would appear that the water content of these lungs may have been somewhat higher than in other groups. The suggestion is that there was a mild edema; however, it should be emphasized that there were no evident changes in lung volumes or pulmonary function, nor were there any apparent structural changes which would confirm this hypothesis.

Liver weights, adjusted for body weight, of both sexes, in the group exposed to 1.5 mg diesel fuel aerosol/L and sacrificed immediately postexposure, were significantly elevated over their sham exposed counter parts. This was contrary to observations in the Phase 2 studies, however, results reported here were adjusted for body weight whereas those reported for Phase 2 did not have this adjustment made. Since there were significant decrements in body weight as a result of diesel fuel aerosol exposure it is possible that this alone would account for the livers of exposed animals appearing to be significantly larger. This hypothesis is to a certain extent supported by the fact that after a two month recovery period all livers, adjusted for body weight, appeared to be very comparable within sex groups.

There were few statistically significant changes in clinical chemistry. Of those that were observed a depression in LDH, cholesterol and creatinine levels in females exposed to 1.5 mg diesel fuel aerosol/L and tested immediately postexposure are potentially of most biological significance. A decrease in serum cholesterol can be evidence of an acute hepatitis and since the decrease in females was aerosol concentration dependent this might do much to explain the observed decrease in serum cholesterol. There were, however, no associated liver lesions that would support this hypothesis and the cholesterol levels can probably be ascribed to decreased food intake and lower body weight. There are no known causes for a decrease in either plasma creatinine or LDH levels; thus the decreases observed in these experiments while, having statistical significance, probably have no biologic importance.

The overall aim of the studies reported here was to determine whether there was any evidence to suggest that multiple exposures of rats to low concentrations of diesel fuel aerosol could cause an evident cumulative toxicity. Although tests were conducted at levels that affected body weight, it is evident, from the data presented in this report, that there was no significant cumulative toxicity that may be attributed to the diesel fuel aerosol. However, even at the concentrations used there is some evidence to suggest that acute CNS disturbance may be occurring which, in an experimental setting, is manifested by a slight decrement in response to the startle reflex. Although no direct extrapolations can be made from rat to man, and these studies were not designed to reveal effects that would be manifested over a lifetime of exposure, it may be concluded from these studies that the inhalation of diesel fuel aerosol is probably an acutely irritating experience but that there is little likelihood of any lasting manifestations provided that exposure is not an extremely frequent and long term event.

Should more work on the toxic potential of the visual obscurant generated from diesel fuel be considered, there are a number of avenues that might be explored. Since there is some evidence of at least transitory effect in the CNS a more detailed study of this aspect might be in order. Approaches might include the effects of long term exposures on behavior parameters such as learning ability and/or in vitro studies of the CNS.

At concentrations used in a previous (Phase II) study, pulmonary effects were observed. Those effects were not noted during the present studies, however, if exposure had been more frequent and longer in duration would changes have been in evidence after 13 weeks? This is a question whose answer might be of value.

LITERATURE CITED

1. Dalbey, W., and S. Lock. 1982. Inhalation Toxicology of Diesel Fuel Obscurant Aerosol in Sprague-Dawley Rats. Final Report, Phase 1, Acute Exposures. Army Project Orders No 9600 and 0027. Oak Ridge National Laboratory, Oak Ridge, TN. AD A132650.

2. MacFarland, H. N. 1976. Respiratory toxicology. In Essays in Toxicology, Vol. 7, W. J. Hayes, Jr., ed., pp 131-153. Academic Press, NY.

3. Finney, D. J. 1971. Statistical Methods in Biological Assay, Second Edition, Griffin Press, London.

4. Dalbey, W., and S. Lock. 1982. Inhalation Toxicology of Diesel Fuel Obscurant Aerosol in Sprague-Dawley Rats. Final Report, Phase 2, Repeated Exposures. Army Project Orders No 0027 and 2802. Oak Ridge National Laboratory, Oak Ridge, TN. AD A142540.

5. Dalbey, W. E., S. Lock, R. Holmberg, J. H. Moneyhun, R. A. Jenkins, and M. R. Guerin. 1981. Toxicologic evaluation of an aerosol of diesel fuel #2. In Proceedings of the 11th Conference on Environmental Toxicology, Wright-Patterson Air Force Base, Dayton, OH, November, 1980. pp 220-231, AFAMRL-TR-80-125.

6. Epstein, R. A., M. A. F. Epstein, G. G. Haddad and R. B. Mellins. 1980. Practical implementation of the barometric method for measurement of tidal volume. J. Appl. Physiol. 49: 1107-1115.

7. Amdur, M. O., and J. Mead. 1958. Mechanics of respiration in unanesthetized guinea pigs. Amer. J. Physiol. 192: 364-368.

8. Raub, J. A., R. R. Mercer, F. J. Miller, J. A. Graham, and J. J. O'Neil. 1982. Dose response of elastase-induced emphysema in hamsters. Am. Rev. Resp. Dis. 125: 432-435.

9. Comroe, J. H., R. E. Forster, A. B. DuBois, W. A. Briscoe, and E. Carlsen. 1962. The Lung. Clinical Physiology and Pulmonary Function Tests. Yearbook Medical Publishers, Chicago.

10. Diamond, L., and M. O'Donnell. 1977. Pulmonary mechanics in normal rats. J. Appl. Physiol. 43: 942-948.

11. Lai, Y.-L., and J. Hildebrandt. 1978. Respiratory mechanics in the anesthetized rat. J. Appl. Physiol. 45: 255-260.

12. Young, J. T. 1981. Histopathological examination of the rat nasal cavity. Fund. Appl. Toxicol. 1: 309-312.

13. Scheffé, H. 1959. The Analysis of Variance. John Wiley and Sons, New York.

14. Lock, S. and W. E. Dalbey. 1984. A method for studying startle reflex in rats and its application to study effects of diesel fuel aerosol. Fund. Appl. Toxicol. (submitted).

APPENDIX

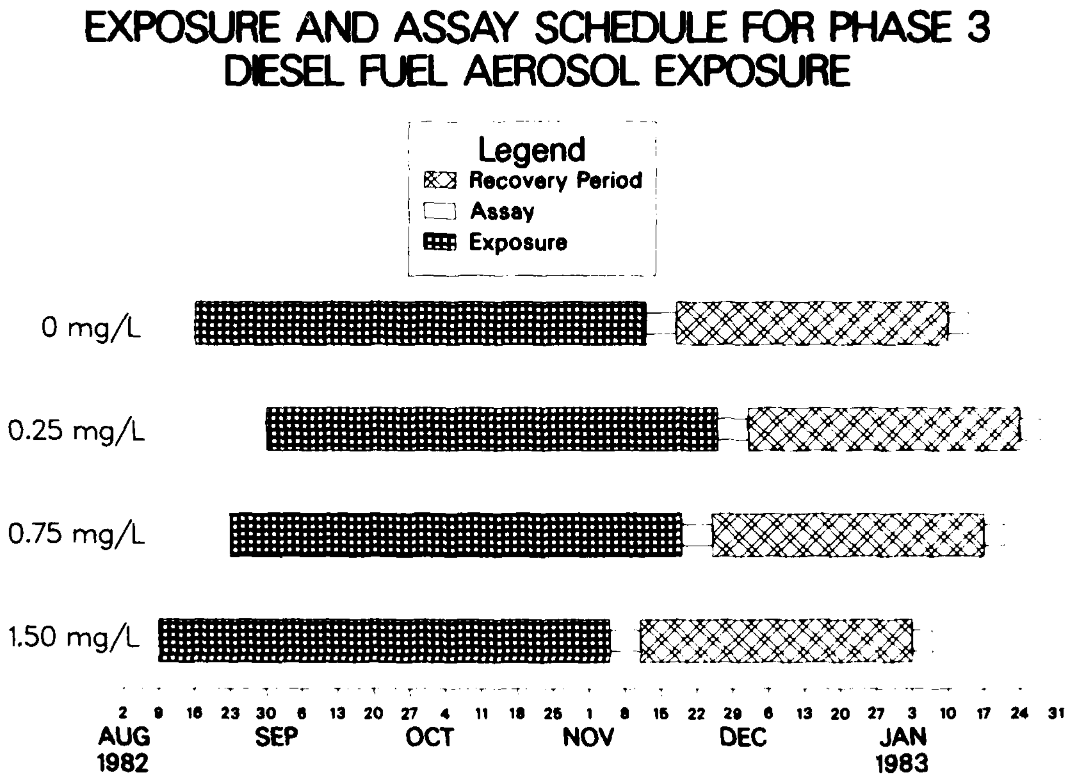
EXPOSURE AND ASSAY SCHEDULE FOR PHASE 3
DIESEL FUEL AEROSOL EXPOSURE
Legend
Recovery Period
Assay
Exposure
0 mg/L
0.25 mg/L
0.75 mg/L
1.50 mg/L
2 9 16 23 30 6 13 20 27 4 11 18 25 1 8 15 22 29 6 13 20 27 3 10 17 24 31
AUG
1982
SEP
OCT
NOV
DEC
JAN
1983

Figure 1A

TABLE 1A. AGE OF ANIMALS IN WEEKS AT DIFFERENT POINTS IN THE EXPOSURE AND POSTEXPOSURE TIMETABLE

Specific Time Point	Vivarium Control	Sham Exposed Control	Diesel Aerosol Concentration		
			0.25 mg/L	0.75 mg/L	1.50 mg/L
Pre-exposure	18.5	18.5	20.5	19.5	17.5
Measurements (Weighing #1)	(weight only)				
First Exposure (Weighing #2)	19	19	21	20	18
Fourteenth Exposure	-	25.5	27.5	26.5	24.5
Twenty-sixth Exposure	-	31.5	33.5	32.5	30.5
Kill Immediately Postexposure (Weighing #15)	32	32	34	33	31
One Month Recovery (Weighing #19)	36	36	38	37	35
Kill After Two Months Recovery (Weighing #23)	40	40	42	41	39

TABLE 2A. ANIMAL UTILIZATION DURING AND AFTER EXPOSURE TO DIESEL FUEL AEROSOL IN THE PHASE 3 STUDY

Time Point	Assay	Animal Numbers
Before exposure #1, #14 and #26	Breathing Frequency and Startle Reflex	13 - 24 M[a] 13 - 24 F
After exposure #1, #14 and #26	Breathing Frequency and and Startle Reflex	13 - 24 M
After odd numbered exposures and weekly during the recovery period	Body weight	13 - 24 M 13 - 24 F (1 - 12 M)[b] (1 - 12 F)[b]
After odd numbered exposures	Body weight	1 - 12 M 1 - 12 F
After odd numbered exposures and weekly during the recovery period	Food consumption	19 - 24 M 19 - 24 F (1 - 6 M)[b] (1 - 6 F)[b]
Four days after the last exposure	Lung lavages and clinical chemistry	9 - 12 M 9 - 12 F
Five and six days after last exposure	Pulmonary function and necropsy for histopathology. Clinical chemistry	1 - 8 M 1 - 8 F 1 - 4 M 1 - 4 F
One month post-exposure	Breathing Frequency and Startle Reflex	13 - 24 M 13 - 24 F
Two month post-exposure	Breathing Frequency and Startle Reflex	13 - 24 M 13 - 24 F
Two months after the last exposure	Lung lavages and clinical chemistry	21 - 24 M 21 - 24 F
Five and six days after last exposure	Pulmonary function and necropsy for histopathology. Clinical chemistry	13 - 24 M 13 - 24 F 13 - 20 M 13 - 20 F

a. Groups used in this study were designated in the following way: RA (1.5 mg/L), SA (sham exposed controls), TA (0.75 mg/L), UA (0.25 mg/L). Group VA (vivarium controls) were not used in any of the assays but were used in the body weight and food consumption studies. A typical animal identification would have been RA-CR21M or UA-CR08F.

b. Group VA (vivarium controls) only.

TABLE 3A. BODY WEIGHT SUMMARY FOR MALE SPRAGUE-DAWLEY RATS OVER A PERIOD OF TWENTY-FIVE WEEKS[a]

Week	Vivarium Controls	Sham Exposed Controls	Diesel Fuel Aerosol Concentration		
			0.25 mg/L	0.75 mg/L	1.5 mg/L
1	509.9 ± 6.4	508.7 ± 9.2	512.6 ± 9.4	510.3 ± 11.7	507.6 ± 7.1
2	517.6 ± 6.8	498.5 ± 8.9	485.1 ± 9.0	487.8 ± 10.8	483.3 ± 6.6
3	529.2 ± 7.3	502.8 ± 8.6	483.1 ± 9.2	479.2 ± 10.9	475.2 ± 6.7[b]
4	535.5 ± 7.3	503.7 ± 8.3[c]	480.1 ± 8.9	474.6 ± 10.8[b]	470.9 ± 6.4[b]
5	545.6 ± 7.1	514.8 ± 8.8[c]	484.5 ± 8.9[b]	473.9 ± 11.0[b]	472.0 ± 6.7[b]
6	553.8 ± 6.8	515.3 ± 8.8[c]	487.6 ± 8.7[b]	475.3 ± 11.0[b]	476.3 ± 6.8[b]
7	558.8 ± 6.9	521.8 ± 8.9[c]	489.7 ± 9.4[b]	480.9 ± 11.4[b]	476.0 ± 7.1[b]
8	565.5 ± 7.6	525.7 ± 8.9[c]	494.4 ±10.0[b]	483.1 ± 11.7[b]	476.5 ± 7.3[b]
9	574.9 ± 7.6	534.8 ± 9.8[c]	499.6 ±10.1[b]	485.9 ± 11.3[b]	481.0 ± 7.3[b]
10	581.0 ± 7.2	540.5 ± 0.1[c]	506.2 ± 9.7[b]	488.9 ± 11.9[b]	486.9 ± 7.7[b]
11	588.0 ± 7.4	548.1 ±10.4[c]	511.5 ± 9.7[b]	492.0 ± 11.8[b]	492.2 ± 7.9[b]
12	594.7 ± 7.5	553.1 ±10.8[c]	514.4 ±10.1[b]	493.2 ± 12.2[b]	495.9 ± 8.0[b]
13	599 1 ± 8.2	559.8 ±11.4[c]	518.5 ±10.2[b]	497.7 ± 12.6[b]	500.5 ± 7.9[b]
14	606.3 ± 8.1	562.6 ±11.7[c]	524.0 ±10.5[b]	498.9 ± 12.8[b]	501.5 ± 8.4[b]
15[d]	606.9 ± 8.4	580.3 ±12.2	543.7 ±10.9[b]	525.6 ± 13.6[b]	528.1 ± 8.8[b]
16	615.3 ± 8.5	578.6 ±19.8	570.5 ±12.9	526.6 ± 18.6[b]	539.6 ±14.0
17	618.0 ± 7.9	586.5 ±20.2	582.6 ±12.5	538.5 ± 19.6[b]	559.1 ±14.4
18	623.6 ± 9.1	593.4 ±20.0	593.1 ±12.3	551.2 ± 19.5	561.6 ±15.0
19	624.6 ±10.3	593.8 ±20.5	590.6 ±12.0	551.7 ± 19.5	566.1 ±14.6
20	639.6 ±10.0	605.3 ±21.5	598.8 ±12.7	557.6 ± 20.1[b]	577.6 ±15.3
21	637.6 ±10.6	608.1 ±21.8	604.6 ±13.3	562.4 ± 19.7	585.6 ±15.5
22	639.6 ±10.7	608.9 ±22.5	608.8 ±13.4	568.8 ± 20.1	590.3 ±16.6
23	641.8 ±11.4	606.1 ±22.9	608.4 ±13.9	570.0 ± 20.7	588.4 ±17.4

a. The weighing on week 1 was carried out before the first exposure. The second weighing was after one exposure.

b. Significantly different from sham exposed controls and vivarium controls ($p < 0.05$).

c. Significantly different from vivarium controls ($p < 0.05$).

d. The fourteenth weighing was carried out immediately after the 25th exposure and the fifteenth weighing was carried out 3 days after the 26th exposure. All weighings after the fifteenth were carried out on 12 animals only.

TABLE 4A. BODY WEIGHT SUMMARY FOR FEMALE SPRAGUE-DAWLEY RATS OVER A PERIOD OF TWENTY-FIVE WEEKS[a]

Week	Vivarium Controls	Sham Exposed Controls	Diesel Fuel Aerosol Concentration 0.25 mg/L	0.75 mg/L	1.5 mg/L
1	279.1 ± 5.7	284.6 ± 3.5	287.7 ± 4.0	266.0 ± 3.7[b]	267.4 ± 3.7[b]
2	283.1 ± 5.7	277.8 ± 3.7	271.9 ± 3.8	254.6 ± 3.2[b]	255.6 ± 3.7[b]
3	289.2 ± 5.7	279.9 ± 3.4	266.4 ± 3.7[b]	250.1 ± 3.2[b]	253.0 ± 3.5[b]
4	287.5 ± 6.7	282.7 ± 4.0	264.9 ± 3.5[b]	251.1 ± 3.2[b]	251.3 ± 3.8[b]
5	293.3 ± 6.6	289.0 ± 4.3	267.5 ± 3.7[b]	249.8 ± 3.5[b]	252.3 ± 3.8[b]
6	296.4 ± 6.7	291.9 ± 3.8	267.4 ± 3.9[b]	251.4 ± 3.3[b]	254.5 ± 3.8[b]
7	301.7 ± 7.1	295.5 ± 3.9	269.9 ± 3.4[b]	251.9 ± 3.4[b]	254.7 ± 3.8[b]
8	304.2 ± 6.5	298.9 ± 4.1	272.6 ± 3.6[b]	255.7 ± 3.4[b]	257.2 ± 3.7[b]
9	306.6 ± 7.8	300.6 ± 4.6	275.6 ± 3.7[b]	255.8 ± 3.2[b]	257.4 ± 3.9[b]
10	308.3 ± 7.2	308.5 ± 4.6	277.9 ± 4.1[b]	259.4 ± 3.3[b]	263.1 ± 3.7[b]
11	313.1 ± 7.7	311.8 ± 5.0	279.9 ± 4.4[b]	259.4 ± 3.3[b]	263.2 ± 3.6[b]
12	317.7 ± 7.6	315.0 ± 5.3	279.1 ± 4.4[b]	261.9 ± 3.3[b]	266.1 ± 3.5[b]
13	316.5 ± 8.1	319.8 ± 5.4	283.6 ± 4.7[b]	262.9 ± 3.6[b]	268.4 ± 3.8[b]
14	317.8 ± 8.6	322.1 ± 5.5	286.6 ± 5.0[b]	267.3 ± 3.9[b]	270.6 ± 4.3[b]
15[c]	320.5 ± 9.3	331.5 ± 6.1	301.3 ± 5.1[b]	279.4 ± 4.2[b]	283.5 ± 4.3[b]
16	324.3 ± 9.5	330.6 ± 8.9	303.1 ± 8.1[b]	281.6 ± 6.3[b]	290.1 ± 6.9[b]
17	325.6 ± 8.1	335.6 ± 9.1	310.4 ± 8.5	284.1 ± 6.5[b]	301.0 ± 8.1[b]
18	328.6 ± 9.2	337.6 ± 9.5	320.3 ±10.4	291.5 ± 7.2[b]	302.1 ± 8.2[b]
19	329.6 ± 9.7	334.1 ±10.0	316.9 ±10.6	294.6 ± 7.1[b]	302.6 ± 7.9[b]
20	337.5 ± 9.3	342.0 ± 9.6	322.6 ±11.0	297.1 ± 8.0[b]	314.1 ± 9.2[b]
21	338.0 ± 9.4	343.2 ±10.1	330.4 ±11.7	303.9 ± 9.0[b]	316.7 ± 9.3
22	339.2 ±10.1	340.9 ± 9.7	336.3 ±12.9	310.6 ± 9.8[b]	323.0 ± 9.2
23	336.8 ±10.0	336.5 ± 9.9	336.3 ±12.1	311.2 ± 9.4	321.4 ± 9.7

a. The weighing on week 1 was carried out before the first exposure. The second weighing was after one exposure.

b. Significantly different from sham exposed controls and vivarium controls ($p < 0.05$).

c. The fourteenth weighing was carried out immediately after the 25th exposure and the fifteenth weighing was carried out 3 days after the 26th exposure. All weighings after the fifteenth were carried out on 12 animals only.

TABLE 5A. FOOD CONSUMPTION (g/day) SUMMARY FOR MALE SPRAGUE-DAWLEY RATS OVER A PERIOD OF TWENTY-ONE WEEKS (Mean ± SEM)

Week	Vivarium Controls	Sham Exposed Controls	Diesel Fuel Aerosol Concentration		
			0.25 mg/L	0.75 mg/L	1.50 mg/L
1[a]	. .	25.92 ± 1.15	27.60 ± 0.84	24.71 ± 1.40	24.92 ± 1.43
2	. .	25.73 ± 1.09	27.52 ± 1.11	23.37 ± 1.60	24.42 ± 0.88
3	. .	27.87 ± 1.21	27.48 ± 0. 9	24.19 ± 1.95[b]	26.02 ± 0.74
4	. .	29.69 ± 1.68	29.42 ± 0.52	24.47 ± 1.35[b]	26.43 ± 0.61[b]
5	. .	27.55 ± 0.76	27.59 ± 1.49	25.26 ± 1.58	24.91 ± 0.86
6	. .	29.83 ± 1.28	27.40 ± 2.69	26.40 ± 2.01	26.19 ± 0.81
7	30.49 ± 0.94	27.90 ± 1.25	29.71 ± 0.94	26.61 ± 2.17	26.40 ± 0.70
8	30.85 ± 0.69	30.40 ± 1.50	30.92 ± 1.61	26.35 ± 1.74[b]	27.33 ± 0.61
9	30.92 ± 1.10	30.85 ± 2.04	31.33 ± 1.05	26.21 ± 1.69[b]	27.49 ± 0.75
10	30.57 ± 1.43	30.33 ± 1.52	30.47 ± 0.66	27.26 ± 2.52	27.97 ± 0.81
11	31.26 ± 1.26	31.12 ± 1.67	30.50 ± 0.32	26.64 ± 1.86[b]	27.57 ± 0.75[b]
12	31.36 ± 1.24	33.26 ± 1.44	31.62 ± 0.81	27.45 ± 1.87[b]	27.52 ± 0.85[b]
13[c]	31.31 ± 0.96	30.83 ± 1.25	30.26 ± 0.47	29.71 ± 2.59	28.48 ± 1.07
14	31.10 ± 0.94	31.05 ± 1.21	33.16 ± 0.54	31.02 ± 2.47	30.90 ± 1.30
15	29.93 ± 1.46	31.90 ± 1.35	34.31 ± 0.59	32.90 ± 2.46	32.88 ± 1.23
16	31.69 ± 1.85	32.83 ± 1.35	34.17 ± 0.63	33.50 ± 2.62	33.00 ± 1.11
17	31.24 ± 1.71	33.33 ± 1.22	33.48 ± 0.42	31.85 ± 2.31	33.57 ± 1.34
18	32.31 ± 1.28	30.55 ± 1.39	32.31 ± 0.50	30.73 ± 2.08	32.16 ± 1.17
19	31.55 ± 1.37	31.19 ± 1.17	32.45 ± 0.75	31.14 ± 2.25	32.17 ± 1.21
20	31.26 ± 1.57	30.24 ± 1.65	32.74 ± 0.74	30.67 ± 2.21	33.14 ± 1.70
21	32.00 ± 1.81	31.24 ± 1.32	35.04 ± 2.29	31.02 ± 2.06	31.59 ± 2.93

a. The first food consumption recorded was one week after the first exposure and three days after the second exposure. Food consumption was always recorded on a Monday.

b. Significantly different from sham exposed control group ($p < 0.05$).

c. The thirteenth measurement of food consumption was made three days after the last exposure.

TABLE 6A. FOOD CONSUMPTION (g/day) SUMMARY FOR FEMALE SPRAGUE-DAWLEY RATS OVER A PERIOD OF TWENTY-ONE WEEKS (Mean ± SEM)

Week	Vivarium Controls	Sham Exposed Controls	Diesel Fuel Aerosol Concentration 0.25 mg/L	0.75 mg/L	1.50 mg/L
1[a]	. .	18.85 ± 0.84	17.62 ± 0.21	18.37 ± 0.33	17.43 ± 0.52
2	. .	17.85 ± 0.94	17.30 ± 0.31	18.27 ± 0.50	17.19 ± 0.63
3	. .	19.37 ± 0.97	18.88 ± 0.65	17.89 ± 0.72	17.61 ± 0.73
4	. .	19.50 ± 0.90	18.26 ± 0.28	18.74 ± 0.87	17.85 ± 0.96
5	. .	20.29 ± 0.91	18.59 ± 0.37	18.02 ± 0.95	17.19 ± 0.88[b]
6	. .	20.85 ± 1.20	19.50 ± 0.57	18.42 ± 0.90	18.57 ± 0.92
7	21.52 ± 0.87	20.14 ± 1.09	19.52 ± 0.55	18.57 ± 0.67	18.35 ± 0.83
8	21.21 ± 0.84	21.90 ± 1.40	20.14 ± 0.57	19.40 ± 0.53	18.87 ± 1.01
9	22.28 ± 0.81	22.07 ± 1.49	19.67 ± 0.53	19.33 ± 0.70	18.71 ± 1.03
10	22.11 ± 0.93	21.85 ± 1.19	19.27 ± 0.45	19.55 ± 0.48	19.19 ± 1.09
11	22.00 ± 0.92	22.50 ± 1.30	19.90 ± 0.48	19.60 ± 0.77	19.52 ± 1.29
12	21.52 ± 0.48	22.33 ± 1.36	20.59 ± 0.59	20.26 ± 0.67	19.55 ± 1.35
13[c]	22.40 ± 0.57	22.23 ± 1.64	20.09 ± 0.83	19.88 ± 0.52	18.90 ± 1.03
14	22.62 ± 0.99	22.33 ± 1.63	21.31 ± 0.75	21.38 ± 0.74	22.26 ± 1.34
15	23.12 ± 0.64	23.85 ± 1.08	22.48 ± 0.93	21.78 ± 0.69	22.90 ± 1.43
16	23.14 ± 1.11	23.31 ± 1.09	23.38 ± 1.18	22.76 ± 0.66	22.90 ± 1.45
17	22.95 ± 0.93	24.48 ± 1.67	24.52 ± 1.35	23.31 ± 0.90	24.14 ± 1.92
18	23.07 ± 1.02	22.17 ± 0.84	22.64 ± 1.18	23.55 ± 0.67	23.64 ± 1.70
19	23.90 ± 0.78	22.31 ± 0.76	23.33 ± 1.25	23.19 ± 0.86	23.07 ± 1.64
20	23.36 ± 0.76	22.43 ± 1.06	23.72 ± 1.16	23.14 ± 0.78	24.69 ± 1.66
21	23.43 ± 0.71	23.38 ± 0.99	23.14 ± 0.95	23.83 ± 0.66	23.52 ± 1.57

a. The first food consumption recorded was one week after the first exposure and three days after the second exposure. Food consumption was always recorded on a Monday.

b. Significantly different from sham exposed control group ($p < 0.05$).

c. The thirteenth measurement of food consumption was made three days after the last exposure.

TABLE 7A. FOOD CONSUMPTION (g/day) SUMMARY FOR SPRAGUE-DAWLEY RATS (SEXES COMBINED) OVER A PERIOD OF TWENTY-ONE WEEKS (Mean ± SEM)

Week	Vivarium Controls	Sham Exposed Controls	Diesel Fuel Aerosol Concentration 0.25 mg/L	0.75 mg/L	1.50 mg/L
1[a]	. .	22.39 ± 1.27	22.61 ± 1.56	21.54 ± 1.18	21.18 ± 1.34
2	. .	21.79 ± 1.37	22.41 ± 1.64	20.82 ± 1.11	20.81 ± 1.21
3	. .	23.62 ± 1.48	23.18 ± 1.38	21.04 ± 1.37[b]	21.82 ± 1.36
4	. .	24.59 ± 1.79	23.84 ± 1.71	21.61 ± 1.16[b]	22.14 ± 1.40[b]
5	. .	23.92 ± 1.23	23.09 ± 1.54	21.64 ± 1.40[b]	21.05 ± 1.30[b]
6	. .	25.34 ± 1.59	23.45 ± 1.77	22.41 ± 1.60[b]	22.38 ± 1.29[b]
7	26.01 ± 1.48	24.02 ± 1.41	24.61 ± 1.62	22.59 ± 1.63	22.38 ± 1.32
8	26.03 ± 1.54	26.15 ± 1.61	25.53 ± 1.82	22.88 ± 1.36[b]	23.10 ± 1.39[b]
9	26.60 ± 1.46	26.46 ± 1.79	25.50 ± 1.85	22.77 ± 1.35[b]	23.10 ± 1.46[b]
10	26.34 ± 1.51	26.09 ± 1.57	24.87 ± 1.73	23.41 ± 1.69[b]	23.58 ± 1.48
11	26.63 ± 1.58	26.81 ± 1.65	25.20 ± 1.62	23.12 ± 1.43[b]	23.54 ± 1.41[b]
12	26.44 ± 1.61	27.80 ± 1.90	26.11 ± 1.73	23.86 ± 1.44[b]	23.54 ± 1.42[b]
13[c]	26.86 ± 1.44	26.53 ± 1.63	25.18 ± 1.60	24.80 ± 1.95	23.69 ± 1.61[b]
14	26.86 ± 1.43	26.69 ± 1.63	27.24 ± 1.84	26.20 ± 1.90	26.58 ± 1.58
15	26.52 ± 1.28	27.88 ± 1.47	28.39 ± 1.86	27.34 ± 2.07	27.89 ± 1.75
16	27.42 ± 1.65	28.07 ± 1.66	28.77 ± 1.75	28.13 ± 2.07	27.95 ± 1.75
17	27.10 ± 1.56	28.90 ± 1.66	29.00 ± 1.51	27.58 ± 1.75	28.86 ± 1.81
18	27.69 ± 1.60	26.36 ± 1.48	27.48 ± 1.58	27.14 ± 1.50	27.90 ± 1.62
19	27.73 ± 1.38	26.75 ± 1.50	27.89 ± 1.54	27.17 ± 1.66	27.62 ± 1.68
20	27.31 ± 1.48	26.33 ± 1.50	28.23 ± 1.51	26.90 ± 1.59	28.92 ± 1.71
21	27.71 ± 1.59	27.31 ± 1.42	29.09 ± 2.15	27.43 ± 1.50	27.56 ± 2.00

a. The first food consumption recorded was one week after the first exposure and three days after the second exposure. Food consumption was always recorded on a Monday.

b. Significantly different from sham exposed control group ($p < 0.05$).

c. The thirteenth measurement of food consumption was made three days after the last exposure.

TABLE 8A. BREATHING FREQUENCY (min^{-1}) OF MALE RATS DURING THE COURSE OF THE PHASE 3 DIESEL FUEL AEROSOL STUDIES

Time Period	Control	Aerosol Concentration 0.25 mg/L	0.75 mg/L	1.50 mg/L
Pretreatment	150.17±6.24	135.82±6.11	147.25±6.13	146.25±6.68
Before 14th Exposure	141.13±6.07	140.00±8.01	138.50±8.84	134.60±7.13
Before 26th Exposure	140.00±4.12	132.54±7.09	138.75±4.37	135.00±7.60
One Month Recovery	132.25±3.85	136.50±5.76	133.67±5.84	126.75±3.88
Two Months Recovery	126.75±5.21	128.50±7.12	126.75±6.42	125.00±4.82

TABLE 9A. BREATHING FREQUENCY (min^{-1}) OF FEMALE RATS DURING THE COURSE OF THE PHASE 3 DIESEL FUEL AEROSOL STUDIES

Time Period	Control	Aerosol Concentration 0.25 mg/L	0.75 mg/L	1.50 mg/L
Pretreatment	118.17±7.28	138.75±5.44	126.75± 6.61	121.68±7.63
Before 14th Exposure	133.81±4.79	127.63±5.87	129.38±10.26	129.11±7.28
Before 26th Exposure	131.75±4.96	129.00±7.18	123.75± 8.02	134.00±6.75
One Month Recovery	128.75±6.65	121.75±5.16	122.00± 7.95	116.25±6.42
Two Months Recovery	108.75±6.96	122.25±5.52	114.50± 6.23	128.50±6.17

TABLE 10A. CHANGES FROM PRETREATMENT VALUES OF STARTLE REFLEX REACTION TIME (msec) IN MALES OVER THE COURSE OF THE PHASE 3 DIESEL FUEL AEROSOL STUDY (Mean ± SEM)

Time Period	Sham Exposed Controls	Diesel Fuel Aerosol Concentration 0.25 mg/L	0.75 mg/L	1.50 mg/L
Pretreatment[a]	16.57±0.43	16.26±0.47	16.82±0.43	16.60±0.43
After 1st Exposure	1.01±0.46	1.70±0.46	2.38±0.47	2.62±0.46[b]
Before 14th Exposure	0.07±0.42	0.55±0.43	0.13±0.43	-0.12±0.43
After 14th Exposure	0.32±0.42	1.13±0.43	1.78±0.44[b]	1.80±0.43[b]
Before 26th Exposure	0.00±0.47	0.57±0.46	0.19±0.47	-0.74±0.47
After 26th Exposure	0.50±0.44	1.96±0.45[b]	1.02±0.44	2.05±0.46[b]
One Month Recovery	0.65±0.48	1.07±0.48	-0.20±0.47	-0.01±0.48
Two Months Recovery	1.00±0.49	0.87±0.48	0.79±0.49	-0.01±0.48

a. Actual values obtained pretreatment.

b. Significantly different from controls ($p < 0.05$).

TABLE 11A. CHANGES FROM PRETREATMENT VALUES OF STARTLE REFLEX REACTION *TIME (msec) IN FEMALES OVER THE COURSE OF THE PHASE 3 DIESEL* FUEL AEROSOL STUDY (Mean ± SEM)

	Sham Exposed Controls	Diesel Fuel Aerosol Concentration		
		0.25 mg/L	0.75 mg/L	1.50 mg/L
Pretreatment[a]	17.13±0.43	17.04±0.43	16.15±0.44	16.95±0.43
Before 14th Exposure	-1.00±0.43	-0.60±0.43	0.39±0.43	-0.08±0.43
Before 26th Exposure	-1.11±0.47	-0.10±0.47	0.33±0.47[b]	-0.06±0.47
One Month Recovery	-0.49±0.47	0.59±0.47	0.80±0.48	-0.01±0.48
Two Months Recovery	0.28±0.49	0.94±0.49	0.47±0.47	-0.05±0.49

a. Actual values obtained pretreatment.

b. Significantly different from controls (p ± 0.05).

TABLE 12A. CHANGES FROM PRETREATMENT VALUES OF STARTLE REFLEX PEAK TIME (msec) IN MALES OVER THE COURSE OF THE PHASE 3 DIESEL FUEL AEROSOL STUDY (Mean ± SEM)

	Sham Exposed Controls	Diesel Fuel Aerosol Concentration		
		0.25 mg/L	0.75 mg/L	1.50 mg/L
Pretreatment[a]	29.03±0.62	26.52±0.62[b]	26.45±0.62[b]	26.22±0.63[b]
After 1st Exposure	0.65±0.81	1.17±0.82	2.76±0.83	2.53±0.82
Before 14th Exposure	-0.93±0.78	0.53±0.78	1.04±0.78	1.46±0.79[b]
After 14th Exposure	-1.58±0.68	0.60±0.68[b]	3.51±0.71[b]	1.72±0.70[b]
Before 26th Exposure	-1.32±0.76	0.63±0.76	1.22±0.77[b]	0.96±0.76[b]
After 26th Exposure	-0.86±0.85	0.51±0.86	1.61±0.85	3.20±0.88[b]
One Month Recovery	-1.43±0.73	1.73±0.73[b]	0.68±0.73[b]	1.44±0.73[b]
Two Months Recovery	-0.09±0.82	1.25±0.81	1.71±0.83	2.70±0.81[b]

a. Actual values obtained pretreatment.

b. Significantly different from controls ($p < 0.05$).

TABLE 13A. CHANGES FROM PRETREATMENT VALUES OF STARTLE REFLEX PEAK TIME (msec) IN FEMALES OVER THE COURSE OF THE PHASE 3 DIESEL FUEL AEROSOL STUDY (Mean ± SEM)

	Sham Exposed Controls	Diesel Fuel Aerosol Concentration		
		0.25 mg/L	0.75 mg/L	1.50 mg/L
Pretreatment[a]	28.19±0.63	26.69±0.63	25.73±0.65	26.42±0.63
Before 14th Exposure	-0.72±0.79	0.98±0.79	0.98±0.79	4.27±0.78[b]
Before 26th Exposure	-1.20±0.77	-0.52±0.76	1.26±0.77[b]	3.11±0.76[b]
One Month Recovery	-0.72±0.73	0.68±0.73	2.10±0.74[b]	2.35±0.73[b]
Two Months Recovery	-0.70±0.86	1.64±0.82	0.90±0.82	1.12±0.82

a. Actual values obtained pretreatment.

b. Significantly different from controls ($p < 0.05$).

TABLE 14A. CHANGES FROM PRETREATMENT VALUES OF STARTLE REFLEX MAXIMUM FORCE EXERTED (g. wt.) IN MALES OVER THE COURSE OF THE PHASE 3 DIESEL FUEL AEROSOL STUDY ADJUSTED FOR BODY WEIGHT (Mean ± SEM)

	Sham Exposed Controls	Diesel Fuel Aerosol Concentration		
		0.25 mg/L	0.75 mg/L	1.50 mg/L
Pretreatment[a]	567±60	601±72	575±60	604±61
After 1st Exposure	-108±41	-222±42	-359±42	-338±41[b]
Before 14th Exposure	39±70	-51±76	-36±63	19±65
After 14th Exposure	-119±54	-163±42	-242±42	-224±42
Before 26th Exposure	5±75	-113±89	-83±76	-34±77
After 26th Exposure	-86±58	-286±46[b]	-246±45	-261±45[b]
One Month Recovery	-1±69	-152±90	-86±74	-49±73
Two Months Recovery	-20±64	-11±88	-29±73	15±70

a. Actual values obtained pretreatment.

b. Significantly different from controls ($p < 0.05$).

TABLE 15A. CHANGES FROM PRETREATMENT VALUES OF STARTLE REFLEX MAXIMUM FORCE EXERTED (g. wt.) IN FEMALES OVER THE COURSE OF THE PHASE 3 DIESEL FUEL AEROSOL STUDY ADJUSTED FOR BODY WEIGHT (Mean ± SEM).

	Sham Exposed Controls	Diesel Fuel Aerosol Concentration		
		0.25 mg/L	0.75 mg/L	1.50 mg/L
Pretreatment[a]	267±117	300±113	202±134	243±131
Before 14th Exposure	110±131	-69±120	-106±139[b]	-40±137
Before 26th Exposure	178±157	58±132	170±156	238±154
One Month Recovery	58±138	-85±127[b]	-22±151	75±149
Two Months Recovery	-113±127	-243±122[b]	-225±144	-191±142

a. Actual values obtained pretreatment.

b. Significantly different from controls ($p < 0.05$).

TABLE 16A. MILLIONS OF ALVEOLAR MACROPHAGES LAVAGED FROM LUNGS DURING PHASE 3 ASSAYS (Mean ± SEM)

Aerosol Conc. (mg/L)	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
0	1.66 ± 0.07	1.60 ± 0.06	1.87 ± 0.55	1.14 ± 0.25
0.25	2.87 ± 0.67	2.18 ± 0.69	1.96 ± 0.42	1.70 ± 0.60
0.75	2.12 ± 0.28	1.54 ± 0.36	1.74 ± 0.12	1.07 ± 0.09
1.50	2.94 ± 0.32	2.02 ± 0.40	1.93 ± 0.27	1.59 ± 0.26

TABLE 17A. LEAST SQUARE MEANS OF THE SQUARE ROOT TRANSFORMATION OF THE NUMBERS OF ALVEOLAR MACROPHAGES (millions) LAVAGED FROM THE LUNGS DURING THE PHASE 3 ASSAYS (Mean ± SEM)

Aerosol Conc. (mg/L)	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
0	1.22 ± 0.18	1.84 ± 0.28	1.23 ± 0.22	1.68 ± 0.30
0.25	1.63 ± 0.15[a]	2.15 ± 0.34	1.30 ± 0.19	1.94 ± 0.32
0.75	1.38 ± 0.18	2.03 ± 0.37	1.25 ± 0.17	1.78 ± 0.35
1.50	1.66 ± 0.15[a]	2.24 ± 0.38	1.29 ± 0.20	1.91 ± 0.31

a. Significantly different from ($p < 0.05$).

TABLE 18A. MILLIONS OF PULMONARY FREE CELLS LAVAGED FROM LUNGS DURING PHASE 3 ASSAYS (Mean ± SEM)

Aerosol Conc. (mg/L)	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
0	2.59 ± 0.29	2.13 ± 0.22	2.20 ± 0.51	1.48 ± 0.21
0.25	3.64 ± 0.92	2.74 ± 0.61	2.50 ± 0.49	2.75 ± 0.58
0.75	2.50 ± 0.32	1.84 ± 0.36	1.87 ± 0.11	1.38 ± 0.12
1.50	3.76 ± 0.37	2.35 ± 0.40	2.23 ± 0.21	1.89 ± 0.25

TABLE 19A. LEAST SQUARE MEANS OF THE SQUARE ROOT TRANSFORMATION OF THE NUMBER OF CELLS OTHER THAN ALVEOLAR MACROPHAGES (millions) LAVAGED FROM THE LUNGS DURING THE PHASE 3 ASSAYS (Mean ± SEM)

Aerosol Conc. (mg/L)	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
0	0.89 ± 0.22	0.43 ± 0.34	0.50 ± 0.26	0.21 ± 0.36
0.25	0.82 ± 0.18	0.41 ± 0.41	0.56 ± 0.23	0.53 ± 0.40
0.75	0.56 ± 0.21	0.18 ± 0.45	0.31 ± 0.21	0.09 ± 0.42
1.50	0.84 ± 0.18	0.20 ± 0.46	0.49 ± 0.24	0.25 ± 0.38

TABLE 20A. PULMONARY RESISTANCE (cm H_2O/mL/sec) OF RATS IN PHASE 3 OF DIESEL FUEL EXPOSURES (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	0.14 ± 0.06	0.08 ± 0.05	0.28 ± 0.06	0.34 ± 0.06
0.25 mg/L	0.08 ± 0.06	0.14 ± 0.06	0.25 ± 0.06	0.22 ± 0.06
0.75 mg/L	0.13 ± 0.06	0.09 ± 0.06	0.14 ± 0.05	0.21 ± 0.06
1.50 mg/L	0.16 ± 0.06	0.14 ± 0.06	0.11 ± 0.06[a]	0.25 ± 0.06

a. Significantly different from control animals ($p < 0.05$).

TABLE 21A. NEGATIVE SLOPES OF LINEAR REGRESSION FOR MULTIBREATH NITROGEN WASHOUT CURVES[a] IN PHASE 3 OF DIESEL FUEL EXPOSURES (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	0.15 ± 0.01	0.09 ± 0.01	0.14 ± 0.01	0.08 ± 0.01
0.25 mg/L	0.18 ± 0.01[b]	0.09 ± 0.01	0.17 ± 0.01[b]	0.12 ± 0.07[b]
0.75 mg/L	0.16 ± 0.01	0.09 ± 0.01	0.17 ± 0.01[b]	0.11 ± 0.01[b]
1.50 mg/L	0.15 ± 0.01	0.08 ± 0.01	0.13 ± 0.01	0.07 ± 0.01

a. y= Log (percent N2).
x = Cumulative dilutions of FRC.

b. Significantly different from control animals ($p < 0.05$).

TABLE 22A. DILUTIONS OF FRC TO OBTAIN 10 PERCENT NITROGEN IN EXPIRED AIR DURING MULTIBREATH NITROGEN WASHOUT IN PHASE 3 OF DIESEL FUEL EXPOSURES (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	4.90 ± 0.38	8.64 ± 0.38	5.90 ± 0.38	9.54 ± 0.43
0.25 mg/L	5.27 ± 0.38	8.85 ± 0.38	4.79 ± 0.38	6.42 ± 0.40[a]
0.75 mg/L	4.96 ± 0.38	8.07 ± 0.38	4.56 ± 0.38[a]	7.37 ± 0.38[a]
1.50 mg/L	5.40 ± 0.38	8.42 ± 0.40	6.34 ± 0.38	10.97 ± 0.40[a]

a. Significantly different from control animals ($p < 0.05$).

TABLE 23A. DILUTIONS OF FRC TO OBTAIN 5 PERCENT NITROGEN IN EXPIRED AIR DURING MULTIBREATH NITROGEN WASHOUT IN PHASE 3 OF DIESEL FUEL EXPOSURES (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	6.85 ± 0.50	12.29 ± 0.50	8.00 ± 0.50	13.20 ± 0.58
0.25 mg/L	7.02 ± 0.50	12.12 ± 0.50	6.53 ± 0.50[a]	8.96 ± 0.53[a]
0.75 mg/L	6.69 ± 0.50	11.34 ± 0.50	6.25 ± 0.50[a]	10.14 ± 0.50[a]
1.50 mg/L	7.49 ± 0.50	12.07 ± 0.53[a]	8.61 ± 0.50	15.19 ± 0.53[a]

a. Significantly different from control animals ($p < 0.05$).

TABLE 24A. SINGLE BREATH CARBON MONOXIDE DIFFUSING CAPACITY (mL/min/mmHg) FOR RATS IN PHASE 3 OF DIESEL FUEL EXPOSURES (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	0.705 ± 0.045	0.350 ± 0.045	0.515 ± 0.045	0.420 ± 0.050
0.25 mg/L	--[a]	--[a]	0.705 ± 0.045[b]	0.400 ± 0.050
0.75 mg/L	0.560 ± 0.045[b]	0.435 ± 0.045	0.565 ± 0.045	0.365 ± 0.045
1.50 mg/L	0.640 ± 0.045	0.380 ± 0.050	0.585 ± 0.045	0.355 ± 0.050

a. No data available.

b. Significantly different from control animals ($p < 0.05$).

TABLE 25A. PEAK FLOW (mL/sec) IN MAXIMAL FORCED EXPIRATORY MANEUVER AMONG RATS IN PHASE 3 OF DIESEL FUEL EXPOSURES (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	48.06 ± 2.28	43.47 ± 2.28	38.89 ± 2.28	36.40 ± 2.63
0.25 mg/L	46.50 ± 2.28	40.91 ± 2.28	33.88 ± 2.27	33.29 ± 2.43
0.75 mg/L	48.44 ± 2.28	39.64 ± 2.28	37.80 ± 2.28	38.86 ± 2.28
1.50 mg/L	47.79 ± 2.28	38.35 ± 2.43	44.93 ± 2.28	36.25 ± 2.44

TABLE 26A. PEAK EXPIRATORY FLOW (mL/sec) DURING ANIMAL FORCED EXPIRATION MANEUVER REFERENCED TO VITAL CAPACITY (mL) (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	2.61 ± 0.17	3.47 ± 0.16	2.13 ± 0.16	2.85 ± 0.18
0.25 mg/L	2.59 ± 0.16	3.35 ± 0.16	1.97 ± 0.15	2.50 ± 0.17
0.75 mg/L	2.84 ± 0.16	3.44 ± 0.16	2.18 ± 0.16	2.92 ± 0.16
1.50 mg/L	2.79 ± 0.16	3.55 ± 0.17	2.53 ± 0.16	2.97 ± 0.17

TABLE 27A. TOTAL LUNG CAPACITY (mL) OF RATS IN PHASE 3 OF DIESEL FUEL EXPOSURES (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	21.03 ± 0.86	13.90 ± 0.81	20.95 ± 0.81	14.53 ± 0.93
0.25 mg/L	20.86 ± 0.81	13.86 ± 0.81	20.06 ± 0.81	14.86 ± 0.86
0.75 mg/L	19.75 ± 0.81	13.07 ± 0.81	19.96 ± 0.81	14.66 ± 0.81
1.50 mg/L	19.76 ± 0.8	12.29 ± 0.86	19.99 ± 0.81	13.44 ± 0.86

TABLE 28A. VITAL CAPACITY (mL) OF RATS IN PHASE 3 OF DIESEL FUEL EXPOSURES (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	18.59 ± 0.80	12.45 ± 0.75	18.26 ± 0.75	12.92 ± 0.86
0.25 mg/L	18.03 ± 0.75	12.30 ± 0.75	17.73 ± 0.75	13.13 ± 0.80
0.75 mg/L	17.08 ± 0.75	11.61 ± 0.75	17.68 ± 0.75	13.45 ± 0.75
1.50 mg/L	17.20 ± 0.75	10.96 ± 0.80	17.79 ± 0.75	12.35 ± 0.80

TABLE 29A. INSPIRATORY CAPACITY (mL) FOR RATS IN PHASE 3 OF DIESEL FUEL EXPOSURES (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	17.26 ± 0.72	11.70 ± 0.68	17.23 ± 0.68	12.03 ± 0.78
0.25 mg/L	16.57 ± 0.68	11.52 ± 0.68	16.08 ± 0.68	12.14 ± 0.72
0.75 mg/L	15.91 ± 0.68	10.81 ± 0.68	16.22 ± 0.68	12.39 ± 0.68
1.50 mg/L	16.05 ± 0.68	10.13 ± 0.72	16.61 ± 0.68	11.61 ± 0.72

TABLE 30A. FUNCTIONAL RESIDUAL CAPACITY (mL) FOR RATS IN PHASE 3 OF DIESEL FUEL EXPOSURES (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	3.77 ± 0.21	2.19 ± 0.20	3.72 ± 0.20	2.49 ± 0.27
0.25 mg/L	4.29 ± 0.20	2.33 ± 0.20	3.98 ± 0.20	2.71 ± 0.21
0.75 mg/L	3.84 ± 0.20	2.26 ± 0.20	3.74 ± 0.20	2.27 ± 0.20
1.50 mg/L	3.71 ± 0.20	2.14 ± 0.21	3.38 ± 0.20	1.83 ± 0.21[a]

a. Significantly different from control animals ($p = 0.005$).

TABLE 31A. RESIDUAL VOLUME (mL) OF RATS IN PHASE 3 OF DIESEL FUEL EXPOSURES (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	2.45 ± 0.17	1.45 ± 0.16	2.69 ± 0.10	1.62 ± 0.18
0.25 mg/L	2.83 ± 0.16	1.56 ± 0.16	2.33 ± 0.16	1.73 ± 0.17
0.75 mg/L	2.67 ± 0.16	1.46 ± 0.16	2.28 ± 0.16	1.21 ± 0.16
1.50 mg/L	2.56 ± 0.16	1.32 ± 0.17	2.19 ± 0.16[a]	1.09 ± 0.17[a]

a. Significantly different from control animals ($p = 0.005$).

TABLE 32A. SPECIFIC COMPLIANCE (mL/cm H_2O/mL) OF RATS IN PHASE 3 OF DIESEL FUEL EXPOSURES (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	1.21 ± 0.09	1.32 ± 0.08	1.15 ± 0.08	1.28 ± 0.09
0.25 mg/L	1.17 ± 0.08	1.26 ± 0.08	1.19 ± 0.08	1.29 ± 0.09
0.75 mg/L	1.11 ± 0.06	1.40 ± 0.08	1.05 ± 0.08	1.20 ± 0.08
1.50 mg/L	1.29 ± 0.08	1.38 ± 0.09	1.22 ± 0.08	1.26 ± 0.09

TABLE 33A. LIVER WEIGHT (g), ADJUSTED FOR BODY WEIGHT, IN ANIMALS SACRIFICED IMMEDIATELY AFTER TWENTY-SIX EXPOSURES TO DIESEL FUEL AEROSOL OR AFTER A TWO MONTH RECOVERY PERIOD (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	10.24 ± 0.55	10.49 ± 0.72	9.67 ± 0.60	11.63 ± 0.65
0.25 mg/L	10.82 ± 0.49	11.12 ± 0.89	11.56 ± 6.3[a]	11.38 ± 0.67
0.75 mg/L	10.72 ± 0.48	10.83 ± 0.47	9.63 ± 0.55	11.17 ± 0.78
1.50 mg/L	13.18 ± 0.51[a]	11.98 ± 0.92[a]	10.61 ± 0.57	11.41 ± 0.74

a. Significantly different from control animals ($p < 0.005$).

TABLE 34A. KIDNEY WEIGHT (g) (LEFT AND RIGHT KIDNEYS AVERAGED), ADJUSTED FOR BODY WEIGHT, IN ANIMALS SACRIFICED IMMEDIATELY AFTER TWENTY-SIX EXPOSURES TO DIESEL FUEL AEROSOL OR AFTER A TWO MONTH RECOVERY PERIOD (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	1.52 ± 0.07	1.09 ± 0.09	1.39 ± 0.07	1.18 ± 0.08
0.25 mg/L	1.64 ± 0.06	1.14 ± 0.11	1.53 ± 0.05	1.15 ± 0.08
0.75 mg/L	1.53 ± 0.06	1.06 ± 0.12	1.53 ± 0.07	1.13 ± 0.09
1.50 mg/L	1.55 ± 0.06	1.13 ± 0.11	1.52 ± 0.07	1.13 ± 0.09

TABLE 35A. SPLEEN WEIGHT (mg), ADJUSTED FOR BODY WEIGHT, IN ANIMALS SACRIFICED IMMEDIATELY AFTER TWENTY-SIX EXPOSURES TO DIESEL FUEL AEROSOL OR AFTER A TWO MONTH RECOVERY PERIOD (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	677 ± 40	618 ± 53	574 ± 44	578 ± 48
0.25 mg/L	664 ± 36	579 ± 65	660 ± 46	546 ± 49
0.75 mg/L	642 ± 35	566 ± 71	585 ± 42	524 ± 55
1.50 mg/L	642 ± 38	628 ± 67	585 ± 42	524 ± 55

TABLE 36A. ADRENAL WEIGHTS (mg), ADJUSTED FOR BODY WEIGHT, IN ANIMALS SACRIFICED IMMEDIATELY AFTER TWENTY-SIX EXPOSURES TO DIESEL FUEL AEROSOL OR AFTER A TWO MONTH RECOVERY PERIOD (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	58 ± 0.7	108 ± 1.0	30 ± 0.8	78 ± 0.9
0.25 mg/L	79 ± 0.7	114 ± 1.2	33 ± 0.9	84 ± 0.9
0.75 mg/L	63 ± 0.6	104 ± 1.3	37 ± 0.7	82 ± 1.0
1.50 mg/L	75 ± 0.7	106 ± 1.2	41 ± 0.8	96 ± 1.0[a]

a. Significantly different from controls ($p < 0.05$).

TABLE 37A. TESTES WEIGHT (g), ADJUSTED FOR BODY WEIGHT, IN ANIMALS SACRIFICED IMMEDIATELY AFTER TWENTY-SIX EXPOSURES TO DIESEL FUEL AEROSOL OR AFTER A TWO MONTH RECOVERY PERIOD (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	3.28 ± 0.12	-	2.65 ± 0.13	-
0.25 mg/L	2.93 ± 0.11	-	3.12 ± 0.84	-
0.75 mg/L	3.20 ± 0.11	-	2.80 ± 0.12	-
1.50 mg/L	3.15 ± 0.12	-	3.09 ± 0.13	-

TABLE 38A. WET WEIGHT OF RIGHT MIDDLE LOBE OF LUNG (mg), ADJUSTED FOR BODY WEIGHT, IN ANIMALS SACRIFICED IMMEDIATELY AFTER TWENTY-SIX EXPOSURES TO DIESEL FUEL AEROSOL OR AFTER A TWO MONTH RECOVERY PERIOD (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	162 ± 8	157 ± 10	143 ± 9	149 ± 9
0.25 mg/L	160 ± 7	161 ± 12	148 ± 9	157 ± 9
0.75 mg/L	173 ± 7	171 ± 14	149 ± 8	144 ± 11
1.50 mg/L	193 ± 7[a]	185 ± 13[a]	148 ± 8	161 ± 11

a. Significantly different from control animals ($p < 0.005$).

TABLE 39A. LUNG WET/DRY RATIO, ADJUSTED FOR BODY WEIGHT, IN ANIMALS SACRIFICED IMMEDIATELY AFTER TWENTY-SIX EXPOSURES TO DIESEL FUEL AEROSOL OR AFTER A TWO MONTH RECOVERY PERIOD (Mean ± SEM)

Exposure Concentration	Immediately Postexposure		Two Months Postexposure	
	M	F	M	F
Controls	5.64 ± 0.19	5.53 ± 0.25	5.04 ± 0.20	4.99 ± 0.23
0.25 mg/L	5.55 ± 0.17	5.35 ± 0.31	5.05 ± 0.22	4.91 ± 0.23
0.75 mg/L	5.36 ± 0.16	5.15 ± 0.33	5.31 ± 0.19	5.01 ± 0.27
1.50 mg/L	5.52 ± 0.17	5.31 ± 0.32	6.95 ± 0.20[a]	7.67 ± 0.26[a]

a. Significantly different from controls ($p < 0.0001$).

TABLE 40A. LEAST SQUARE MEANS OF CLINICAL CHEMISTRY PARAMETERS FOR ALL SHAM EXPOSED ANIMALS IN PHASE 3 OF DIESEL FUEL EXPOSURE (Mean ± SEM)

	MALE	FEMALE
Alkaline Phosphatase	58.83 ± 3.92[a]	24.45 ± 1.83
SGOT	9.37 ± 0.34	9.69 ± 0.49
LDH	13.41 ± 1.16	12.80 ± 1.23
Glucose	181.58 ± 12.85	143.55 ± 4.51
Cholesterol	67.42 ± 3.74	79.64 ± 4.98
Triglyceride	8.27 ± 0.50	7.85 ± 0.52
BUN	17.25 ± 0.68	18.55 ± 0.99
Uric Acid	1.98 ± 0.22	1.52 ± 0.12
Bilirubin	0.25 ± 0.06	0.52 ± 0.07
Sodium	140.17 ± 0.63	138.18 ± 0.48
Potassium	5.92 ± 0.23	5.75 ± 0.35
Creatinine	0.67 ± 0.06	0.74 ± 0.05

a. Groups collapsed across measurement time so that results here represent values combined from immediately postexposure and two months post-exposure.

TABLE 41A. LEAST SQUARE MEANS OF HEMATOLOGICAL PARAMETERS FOR ALL SHAM EXPOSED ANIMALS IN PHASE 3 OF DIESEL FUEL EXPOSURE (Mean ± SEM)

	MALE	FEMALE
RBC (x10)	2.70 ± 0.16[a]	2.83 ± 0.19
WBC (x10)	1.38 ± 0.24	1.23 ± 0.14
Hematocrit (%)	50.13 ± 1.42	48.25 ± 1.20

a. Groups collapsed across measurement time so that results here represent values combined from immediately postexposure and two months post-exposure.

TABLE 42A. SUMMARY OF HISTOPATHOLOGICAL RESULTS

Observation	Sacrificed Immediately Post Treatment[a] Aerosol Concentration (mg/L)				Sacrificed After Two Months Recovery[a] Aerosol Concentration (mg/L)			
	0	0.25	0.75	1.50	0	0.25	0.75	1.50
Pneumonitis, focal	2[b]	4	2	3	2		2	1
Debris in nasolacrimal duct	3	3	4	2	4	1	3	6
Exudate in nasal cavity	1			1				
Glomerulosclerosis	16	16	16	16	16	16	16	16
Adrenal cortical adenoma					2	3[c]	3	0
Myocarditis, focal	1	1	1	4	3	2	3	3
Intestinal nematodiasis	2	2	1		5	2	3	3
Thyroid, parakeratotic follicles	2	1		2			4	
Hydronephrosis			1		1	1		
Pyelonephritis		1			1			
Renal cyst					1	1		
Urinary bladder, cystitis		1						
Urinary bladder, hyperplasia					1			
Glossitis		1			1			
Metritis						1	1	
Ovary, follicular cyst			1					
Mammary, myxoma							1	
Renal adenomatous hyperplasia	1							
Preputial gland abscess			1					
Steatitis				1				
Fat necrosis				1				
Dermatitis, suppurative							1	
Testis, mineralization		1						

a. 16 rats per group.

b. Number of rats with the lesion indicated.

c. One rat had an adenoma in each adrenal.

PERSONNEL

The following personnel received support under Army Project Orders 0027 and 2802 from the U.S. Army Medical Research and Development Command in the performance of the work described in this report:

Principal Investigator	:	Simon Lock, PhD.
Technicians	:	William Klima Fred Stenglein
Statistician	:	Richard Schmoyer, PhD.
Pathologist	:	Richard Griesemer, DVM PhD.

The following personnel from the Analytical Chemistry Division were responsible for aerosol generation and monitoring.

Michael Guerin, PhD.
Robert Holmberg, PhD.
Roger Jenkins, PhD.
Jack Moneyhun

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